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EFFECT OF IODINE ON CHOLESTEROL-INDUCED ATHEROSCLEROSIS

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Although iodine has been used empirically for many years in the treatment of arteriosclerosis in man, experimental proof is lacking that it can alter an arteriosclerotic plaque. There is evidence, however, that when administered in organic or inorganic combination to rabbits fed large amounts of cholesterol, iodine can inhibit, at least partially, the production of the characteristic arterial lesions induced by cholesterol. Until now, however, there has been no study of the effect of iodine on fully developed cholesterol-induced atherosclerosis. In view of the protective power of the drug against atherosclerosis induced by cholesterol and with its effect on infectious granuloma in mind, it seemed reasonable that iodine might accelerate the healing and absorption of cholesterol-induced lesions. Were this true, it would suggest another basis for the utilization of iodine in arteriosclerosis in man.

The procedure used in the study of the possible resorptive effect of iodine on the lesions of experimental atherosclerosis was to produce such lesions by adequate feeding of cholesterol to rabbits and then to feed potassium iodide for various periods.

METHOD OF INVESTIGATION

Twenty-five gray chinchilla rabbits, each weighing 2 Kg., were used. According to the technic of Turner [1] each rabbit was fed 1 Gm. of cholesterol mixed with its food four times a week for one hundred and five days. This period of time and this dosage have been demonstrated to be ample for the regular production of aortic lesions. The feeding of cholesterol was then stopped, and the animals were divided into three groups as follows: (1) Nine rabbits were killed immediately as controls; (2) eight rabbits were allowed to rest on an ordinary stock diet, two of which were killed one month after the cholesterol was discontinued, three two months after and three three months after, and (3) the remaining eight rabbits were placed

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1. Turner, K. B.: J. Exper. Med. **58**:115, 1933.

on the same stock diet and were fed 0.85 Gm. of potassium iodide daily by pipet in the form of 1 cc. of a concentrated solution (60 Gm. of potassium iodide in each 70 cc. of solution). This method of administration proved satisfactory. The rabbits gained weight and remained in good condition. Two of them were killed one month after the cessation of the feeding of cholesterol and the commencement of potassium iodide, three two months after and three three months after. The content of cholesterol in the serum, both the total and free cholesterol, was determined at frequent intervals by the method of Schoenheimer and Sperry.[2]

When examined at the end of the experimental periods all the rabbits presented vascular lesions of the type due to cholesterol. The aortas of the nine rabbits used as controls, which were killed at the end of one hundred and five days of feeding of cholesterol, presented characteristic intimal plaques, varying considerably in extent and number from a few small lesions in the arch to extensive confluent infiltrations throughout the vessel. Similar lesions were not infrequently present in the cusps of the mitral and aortic valves, the larger pulmonary arteries and the main branches of the aorta. In gross appearance and frequency the lesions in the aortas of rabbits fed cholesterol for one hundred and five days and allowed to rest on a stock diet for from one to three months could not be distinguished from those in the first group used as controls. The aortic lesions of rabbits of the third group, those which received potassium iodide after cessation of the cholesterol, differed in no essential respect from those of the other animals. If anything, the lesions in the aortas of these rabbits were somewhat more extensive than those in the animals which were resting on the stock diet. Nor did microscopic study reveal any difference between the lesions in the animals given potassium iodide and those in the animals which received none. Though there was evidence of continuing fibrosis of the cholesterol-induced plaques after the feeding of cholesterol was stopped, there was no real difference in the amount of fibrosis between lesions in the resting animals and those in rabbits fed potassium iodide (table). There was considerable variation in the degree of atherosclerosis within each group, but this could not be ascribed to lack of absorption of cholesterol, for in every animal the cholesterol content of the serum was well above normal, in all but three cases being more than 700 mg. per hundred cubic centimeters of blood, and the content remained high for a considerable, though variable, time after the feeding of cholesterol was stopped. There was, then, no correlation between the extent of sclerosis and the experimental procedure.

COMMENT

The inability to alter fully developed cholesterol-induced arterial lesions with moderately large doses of potassium iodide whereas iodine

2. Schoenheimer, R., and Sperry, W. M.: J. Biol. Chem. **106**:745, 1934.

Values for Cholesterol of the Serum in Rabbits Fed Cholesterol

Rabbit	Length of Experiment, Days*												Length of Time on Potassium Iodide or Resting, Months	Degree of Atherosclerosis
	42		96		112†		134		149		197			
	Total, Mg. per 100 Cc.	Ratio Ester/Free	Total, Mg. per 100 Cc.	Ratio Ester/Free	Total, Mg. per 100 Cc.	Ratio Ester/Free	Total, Mg. per 100 Cc.	Ratio Ester/Free	Total, Mg. per 100 Cc.	Ratio Ester/Free	Total, Mg. per 100 Cc.	Ratio Ester/Free		
With Potassium Iodide														
205	868	3.32	840	2.08	1,184	1.55	761	1.09	...	....	...	....	1	++++
207	535	3.11	251	1.76	346	1.29	449	1.09	...	....	...	....	1	+
202	666	2.66	1,088	3.51	488	2.03	265	2.08	186	1.82	...	....	2	++
203	593	2.87	807	2.49	492	2.64	319	2.04	184	1.92	...	....	2	++
204	542	1.88	1,015	2.80	725	2.44	322	1.87	249	1.77	...	....	2	+
208	402	3.28	874	2.36	611	2.55	718	1.98	465	1.72	93	1.45	3	++
209	376	2.55	875	2.74	721	1.99	467	1.73	203	1.54	164	1.55	3	++++
211	630	2.82	860	2.28	705	2.67	566	2.27	328	1.78	294	2.10	3	+++
Without Potassium Iodide														
206	72	4.15	336	2.57	271	3.11	99	2.00	...	....	...	....	1	+?
213	177	4.06	840	2.84	842	3.15	475	2.60	...	....	...	....	1	+
216	520	3.05	670	3.21	511	2.87	279	2.98	106	2.78	...	....	2	+
217	524	3.40	946	3.36	565	3.01	141	2.86	109	2.76	...	....	2	+
221	801	2.96	920	2.25	880	2.78	403	2.67	264	2.67	...	....	2	+++
218	689	2.49	1,004	2.60	880	2.89	434	2.68	285	2.48	123	1.98	3	++++
219	886	3.12	832	1.20	1,105	1.98	635	1.90	464	1.99	69	2.64	3	++
222	33	2.88	331	3.09	291	2.68	144	2.20	73	2.76	25	3.03	3	+

* Feeding of cholesterol was stopped on the one hundred and fifth day.
† One week after feeding of cholesterol was stopped.

in both organic and inorganic form when given early has the power of preventing the deposition of cholesterol and its esters (Liebig, Seel and Creuzberg, Mori and Shinoi, Turner, and Ungar)[3] points to irreversibility of the atherosclerotic process. Even after many months the lesions remain as fibrous plaques.[4] (There is little doubt, however, that the frequent intimal plaques seen in typhoid fever and other febrile disturbances, especially in childhood, are resorbed.)

The distinction which must apparently be made between cholesterol-induced lesions which are developing and those already formed is further emphasized by a study of the cholesterol of the serum. In the normal rabbit the ratio of the amount of cholesterol esters to that of free cholesterol in the blood serum varies between 1.85 : 1 and 3 : 1. During the period of feeding cholesterol this ratio is generally not appreciably altered, the amounts both of free cholesterol and of cholesterol esters increasing to the same degree. Liebig,[3a] Seel and Creuzberg[3c] and Turner[1] found that administration of iodine with cholesterol resulted in a reduction in the cholesterol content of the serum and in protection against atherosclerosis. (Rosenthal,[5] however, reported an increase in the amount of cholesterol in the blood and in the degree of atherosclerosis when small amounts of iodine were administered [from 2 to 3 mg. of iodine daily], which was in contrast to the findings of Seel and Creuzberg, who also used small doses. The major difficulty in comparing the findings of the several workers in this field is that there is no uniformity of procedure, the amount of cholesterol and of iodine given and the duration of feeding cholesterol, both before and with the administration of iodine, varying from one experiment to another.) It was found in the present study, however, that when iodine was administered after the cessation of feeding of cholesterol there was a progressive decrease in the ratio of the amount of cholesterol esters to free cholesterol as the feeding of potassium iodide continued (table), until after six weeks of administration of potassium iodide the ratio in every case was at or below the lower limit of the normal value, whereas the ratios in the resting animals were significantly higher. This is in contrast to the findings of Rosenthal, namely, that the value for the esters remained high for as long as six weeks when potassium iodide was fed with cholesterol. It was further observed that, although the amount of cholesterol

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3. (*a*) Liebig, H.: Med. Klin. **25**:1100, 1929; Riforma med. **47**:1400, 1931; Arch. f. exper. Path. u. Pharmakol. **159**:265, 1931. (*b*) Mori, K., and Shinoi, K.: Mitt. d. med. Gesellsch. zu Tokio **46**:316, 1932. (*c*) Seel, H., and Creuzberg, G.: Arch. f. exper. Path. u. Pharmakol. **161**:674, 1931. (*d*) Ungar, H.: ibid. **175**: 536, 1934. (*e*) Turner.[1]

4. Anitschkow, N., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, p. 291.

5. Rosenthal, S. R.: Arch. Path. **18**: 827, 1934.

esters in the blood was rapidly decreasing in the rabbits receiving potassium iodide, the total cholesterol content remained relatively high. Six weeks after the feeding of cholesterol was stopped none of the rabbits given potassium iodide had a normal amount of cholesterol in the blood, whereas that of three of the resting group was again within the normal range. Even at the end of three months, the blood of only one of the three remaining animals given potassium iodide had a normal cholesterol content, while that of all three of the remaining resting animals had reached the normal level. This retardation of the return of the cholesterol of the blood to the normal level when potassium iodide was administered is not in harmony with the findings of Seel and Creuzberg, who reported a more rapid decrease in the amount of cholesterol in the blood of the animals fed iodine after twenty-four days of administration of cholesterol. An explanation of the aforementioned differences is not obvious, although both Rosenthal and Seel and Creuzberg administered smaller doses of iodine.

The relatively rapid decrease in the amount of cholesterol esters in the blood serum of the rabbits receiving potassium iodide implies either storage or destruction of the esters. The presence of large amounts of free cholesterol in the blood serum suggests mobilization of stored cholesterol and favors the hypothesis that the esters were destroyed. The mobilization of stored cholesterol under the influence of potassium iodide, if it occurs, not only would account for the retardation of the return of the cholesterol content of the blood to normal levels but might also explain the somewhat greater degree of atherosclerosis in the rabbits which received potassium iodide.

The experiments of Murata and Kataoka,[6] Friedland[7] and Turner and Khayat[8] pointed to the thyroid gland as having a rôle in the prevention of experimental atherosclerosis in rabbits. Examination of the thyroid glands of the rabbits fed cholesterol for one hundred and five days and then either permitted to rest for from one to three months or fed potassium iodide for the same periods failed to reveal consistent differences. Practically all the glands in both groups were composed predominantly of large follicles, lined by flat epithelium and filled with well stained colloid. There was no correlation between the state of the thyroid gland and the degree of atherosclerosis.

SUMMARY

Potassium iodide fed in large doses for from one to three months to rabbits in which atherosclerosis had previously been induced by pro-

6. Murata, M., and Kataoka, S.: Verhandl. jap. path. Gesellsch. **8**:221, 1918.
7. Friedland, I. B.: Ztschr. f. d. ges. exper. Med. **87**:683, 1933.
8. Turner, K. B., and Khayat, G. B.: J. Exper. Med. **58**:127, 1933.

longed feeding of cholesterol does not influence the rate or nature of the involution of the vascular lesions.

Potassium iodide appears to retard the return of the cholesterol content of the blood to normal levels, although it markedly depresses the ratio of the amounts of cholesterol esters and free cholesterol. It is suggested that this retardation may be due to mobilization of stored cholesterol from the tissues.

Dr. W. M. Sperry of the Babies Hospital determined the values for cholesterol.

EXPERIMENTS RELATIVE TO VACCINATION AGAINST TUBERCULOSIS WITH THE CALMETTE-GUÉRIN BACILLUS (B C G)

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During recent years a great deal of interest has been stimulated in the possibility of increasing the protection of children against tuberculosis by vaccinating them with the Calmette-Guérin bovine strain of the tubercle bacillus (B C G). In most cases the vaccine has been administered orally, but recent efforts have been directed toward the use of the vaccine injected as living organisms subcutaneously. This method of giving the vaccine has been used by Heimbeck [1] and by Park and his associates.[2]

The literature on B C G is extensive, and a complete analysis is not attempted in this paper. A comprehensive digest of the literature that appeared up to 1929 was published by Petroff.[3] Since 1929 critical reviews have been written by Bouquet,[4] Kraus [5] and Lurie.[6]

From these reviews it is evident that there is still lack of agreement in regard to the innocuousness of B C G. Most workers seem to agree that for the most part B C G may be looked on as a strain of the tubercle bacillus which has lost its property of producing progressive tuberculosis. Other experimenters have reported that under certain conditions of cultivation, as in deep peptone broth (Dreyer and Vollum [7]), by the addition of normal rabbit serum (Sasano and Medlar [8]) or by growing the organisms in contact with antiserum (Petroff), the strain may become virulent and produce progressive tuberculosis. Numerous experiments have been reported, some seemingly to prove and some to disprove these contentions. The recent work of Park and his associates seems to show fairly definitely that the inoculation of children subcutaneously with large doses of living B C G carries with it no definite danger.

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1. Heimbeck, J.: Arch. Int. Med. **49**:957, 1932.
2. Park, W. H.; Kereszturi, C., and Mishulow, A. B.: J. A. M. A. **101**:1619, 1933.
3. Petroff, S. A.: Am. Rev. Tuberc. **20**:275, 1929.
4. Bouquet, A.: Am. Rev. Tuberc. **24**:764, 1931.
5. Kraus, Rudolf: Am. Rev. Tuberc. **24**:778, 1931.
6. Lurie, M. B.: J. Exper. Med. **60**:163, 1934.
7. Dreyer, G., and Vollum, R. L.: Lancet **1**:9, 1931.
8. Sasano, K. T., and Medlar, E. M.: Am. Rev. Tuberc. **23**:215, 1931.

Calmette [9] observed more than 400 autopsies performed on children who had been vaccinated but had died of other causes. In none of these children did he note evidence of tuberculous lesions.

There is also failure among experimenters to agree in regard to the value of B C G in developing resistance to subsequent infections with virulent bovine or human strains. Most observers agree, however, that a degree of resistance follows vaccination.

In the experiments reported in this paper an effort was made to study quantitatively immune reactions in experimental tuberculosis. Allergy and resistance were studied as related to each other and as each is related to antibodies, agglutinins, complement-fixing antibodies, opsonins and lysins.

The relation of these reactions to the pathogenesis of pulmonary tuberculosis was observed to see whether in a comparative way helpful conclusions could be drawn concerning the pathogenesis of pulmonary tuberculosis in man.

MATERIALS AND METHODS

The strain of B C G used in the experiments was obtained from Feldman [10] and is the one used by him to produce lesions in rabbits. Feldman obtained this strain from Calmette in January 1930. The organism was cultured by Feldman on glycerin-broth-potato medium until October 1930. Subcultures were made every four weeks. From October 1930 until April 1933 the organism was grown with monthly transfers on Feldman's modified glycerinated egg medium. Since April 1933 this strain has been subcultured each month on agar to which was added 0.2 per cent dextrose, veal infusion and 5 per cent glycerin. It has not come in contact with bile since October 1930. Since coming into my possession it has been kept most of the time at 37 C., and large quantities have been grown.

The virulent bovine strain (Ravenel) used in testing resistance in rabbits was supplied by Lurie [11] from the Henry Phipps Institute. Lurie found that when 0.01 mg. of these organisms was injected intravenously into rabbits death from pulmonary and generalized tuberculosis resulted on an average in about forty days. These findings were confirmed in my work. The human strain (H 37) was used in testing resistance in guinea-pigs.

Old tuberculin was used in determining the presence and degree of allergy. One milligram of old tuberculin was injected intracutaneously. The volume injected was 0.1 cc. This amount injected into the skin of the rabbit or guinea-pig produced an excellent wheal. The tuberculin was of such potency that when 0.05 mg. was injected intracutaneously into a person allergic to the tubercle bacillus a strong positive reaction was elicited. Reactions in the rabbits and guinea-pigs were observed forty-eight hours after the injection of the tuberculin. Redness with slight induration was read as +. Extreme redness and induration with necrosis were reported as ++++. The readings ++ and +++ indicated intermediate stages of induration without necrosis. Necrosis in the area of injection was required for a reading of ++++.

9. Calmette, A.: Am. Rev. Tuberc. **27**:1, 1933.

10. Feldman, W. H.: Am. J. Path. **8**:755, 1932.

11. Lurie, M. B.: Personal communication.

The degrees of development of the microscopic lesions observed in the visceral organs of the animals were recorded in grades of from + to ++++. Grade + represented only an occasional area of tuberculous pneumonia or a small, solitary epithelioid tubercle. Grade ++++ represented many tubercles or areas of tuberculous pneumonia scattered thickly throughout the slides. Grades ++ and +++ indicated intermediate stages.

Rabbits weighing from 3 to 4 pounds (1.3 to 1.8 Kg.) were used in studying safety and resistance and guinea-pigs in studying resistance developed to the human strain.

The rabbits in the regular course of vaccination received four injections of 1 mg. of B C G at intervals of one week. Several rabbits, for comparison of the degrees of allergy and resistance, were given more injections or larger amounts, or both. When it was desired to make animals allergic each rabbit was given one injection subcutaneously of from 1 to 4 mg. of living BCG.

The BCG used for vaccination was ground in a mortar into a fine suspension in a salt solution. The suspension had a milky appearance, but particles could not be detected. The organisms were killed by heating in a water bath at 60 C. for thirty minutes. The sterility of the vaccine was confirmed by failure of large quantities of the heated organisms to grow on agar containing veal infusion and glycerin, on which living organisms grew readily.

The four following methods of administering the vaccine were studied and compared: injection of (1) living organisms subcutaneously, (2) living organisms intravenously, (3) heat-killed organisms subcutaneously and (4) heat-killed organisms intravenously.

A series of rabbits were also given injections of living BCG directly into the lung through the trachea by means of a long needle. The purpose of this manner of administering the BCG was to study the comparative effects of allergy and resistance in the pathogenesis of tuberculosis.

The four methods of giving the vaccine were suggested by results obtained from vaccinating rabbits with streptococci. In the experiments on vaccination with streptococci I [12] found that the intravenous method of administering the vaccine proved to be superior to the subcutaneous method in developing a higher degree of resistance, as demonstrated by the greater rate of destruction of organisms in the blood and liver and by a much higher agglutination titer. It was also shown that the intravenous method had an advantage over the subcutaneous in not producing a measurable amount of hypersensitiveness (allergy) to streptococci in the animals given injections and in desensitizing animals already hypersensitive. This greater degree of protection and the absence of allergy applied to both living and heat-killed streptococci when injected intravenously. The guinea-pigs were vaccinated subcutaneously with living organisms only. The amount given at each of the four weekly injections was 0.05 mg.

An attempt was made in the experiments reported in this paper to find the safest method of administering B C G vaccine which would result in the highest degree of resistance. The two chief considerations, therefore, were safety and resistance.

SAFETY

The factors of safety studied in vaccinating animals with B C G were (1) mechanical injuries, such as bacterial emboli in the brain or

12. Clawson, B. J.: J. Infect. Dis. **53**:157, 1933.

other organs; (2) immediate or delayed toxic effects of the vaccine on normal or allergic animals; (3) whether or not allergy resulted and the significance of the allergy, and (4) development of lesions in the visceral organs (lungs, liver, spleen and kidneys) as a result of vaccination.

Mechanical Injuries.—These injuries, if present, would be most likely to be observed in the animals vaccinated intravenously with organisms, either living or heat-killed. The brain, lungs, liver, spleen and kidneys from many animals vaccinated intravenously or subcutaneously and killed for the purpose or dying of other causes were examined microscopically. In none of the organs of the animals were clumps of acid-fast organisms shown, and there was never any change in the tissues which gave evidence of embolism. At no time was there any clinical or morphologic evidence of ill effects from mechanical causes. From the standpoint of mechanical injury vaccination subcutaneously or intravenously with living or heat-killed B C G seemed to be safe.

Toxic Results.—The possibility of toxic results was studied in normal animals and in animals which had been made allergic. In more than 250 normal animals vaccinated by one of the four methods and observed at periods of from a few weeks to as long as five months after the last injection of the vaccine there were no evidences of toxic effects, either immediate or delayed, other than those detected after injection of any other vaccine. The animals continued to be healthy and to grow and gain in weight.

Many animals were given a double dose of vaccine at each injection. Some were given many injections. Several were given intravenous injections of 1 mg. of living B C G at weekly intervals for as long as seventeen weeks. In none of the vaccinated animals were there at any time indications of toxemia due to vaccination.

The absence of toxic effects was observed only in normal animals. Different results were obtained in animals which had been made allergic and then given intravenous injections of B C G, especially in large doses. In the allergic animals marked toxic symptoms were noted in from twelve to twenty-four hours after an intravenous injection of a large dose of living or heat-killed B C G. In some of these animals death occurred (table 4).

Allergy.—Another consideration for safety in vaccinating with B C G is whether an allergic state is developed in the course of vaccination. The term allergy as used in this paper is synonymous with hypersensitiveness to tuberculoprotein. This type of hypersensitiveness or allergy is exhibited as a delayed local reaction at the point of injection or as delayed general shock following an intravenous injection. The local reaction is indicated by redness, swelling and cellular infiltration fol-

lowing an intracutaneous injection of tuberculin and is called the Mantoux reaction. This type of hypersensitiveness was classified as "hypersensitiveness to infection" by Coca and Cooke.[13] Rich and Lewis[14] referred to it as "an inflammatory-necrotizing type of hypersensitiveness to tuberculosis." Zinsser[15] spoke of such hypersensitiveness in general as "bacterial allergy."

The two important things to know about allergy, if it is developed during a course of vaccination with B C G, is whether the allergic state may be dangerous and whether its presence is necessary for resistance. The significance of allergy in the pathogenesis of tuberculosis also has to be considered.

It is now generally believed that necrosis with its harmful results in tuberculous lesions is dependent on the allergic state. Long[16] stated that the opinion is now growing more general that necrosis is largely the effect of the protein of the tubercle bacillus on a body made hypersensitive to the micro-organism by preexisting infection with the bacillus.

Rothschild, Friedenwald and Bernstein,[17] from experiments in desensitizing tuberculous guinea-pigs with tuberculin, concluded that allergy was responsible for necrosis and mechanical spread in tuberculosis.

Birkhaug[18] found that better protection was obtained from vaccinating guinea-pigs with B C G if the animals were not infected with the human strain until the allergy due to vaccination had decreased.

Evidently much harm may result from necrosis in a tuberculous organ. The necrosis can be a means of wide dissemination of the bacilli with spread of the infection. It would seem, therefore, that allergy should be looked on as a harmful factor in tuberculosis and should be avoided in vaccination against tuberculosis if resistance can be developed as well without it.

There is a dispute among immunologists at present as to whether allergy is necessarily a part of the state of resistance in tuberculosis as well as in other diseases. It is commonly stated that without allergy there is no resistance in tuberculosis.

Rich and his associates[19] concluded that it has not yet been proved that an allergic state is a necessary part of the general process of

13. Coca, A. F., and Cooke, R. A.: J. Immunol. **8**:163, 1928.

14. Rich, A. R., and Lewis, M. R.: Bull. Johns Hopkins Hosp. **50**:115, 1932.

15. Zinsser, H.: Bull. New York Acad. Med. **4**:351, 1928.

16. Long, E. R.: Am. Rev. Tuberc. **22**:467, 1930.

17. Rothschild, H.; Friedenwald, J. S., and Bernstein, C.: Bull. Johns Hopkins Hosp. **54**:232, 1934.

18. Birkhaug, K. E.: Am. Rev. Tuberc. **27**:6, 1933.

19. Rich, A. R.; Chesney, A. M., and Turner, T. B.: Bull. Johns Hopkins Hosp. **52**:179, 1933. Rich, A. R., and Brown, J. H.: Proc. Soc. Exper. Biol. & Med. **27**:695, 1930. Rich, A. R.; Jennings, F. B., and Downing, L. M.: Bull. Johns Hopkins Hosp. **53**:172, 1933.

immunity (resistance), tuberculous or otherwise. They showed that resistance in syphilis may be present when the reaction to the luetin test (indicating the allergic state) is negative. They also demonstrated by injecting into normal animals the serum of immune animals which were both resistant and allergic to the pneumococcus that the resistance was transferred to the normal animals while the allergic factor was left behind. Their experiments in desensitizing animals immune and allergic to the pneumococcus or to Bacillus avisepticus and later in demonstrating marked resistance in these desensitized animals also seem to show that allergy is not necessary in the phenomenon of protection. Similar results were obtained by Rothschild, Friedenwald and Bernstein by desensitizing guinea-pigs allergic to tuberculosis.

Branch and Cuff [20] were able by injecting heat-killed tubercle bacilli intravenously or intramuscularly to develop resistance in guinea-pigs to the human tubercle bacillus without associated allergy.

In experiments in vaccination with streptococci I found that general resistance to streptococcic infection could exist without allergy. It was also concluded from these experiments that allergy as related to general resistance in streptococcic infection is a useless and at times a harmful phenomenon.

The opinions for and against the necessity of allergy in the immune state in tuberculosis are illustrated by the writings of Zinsser and Mueller,[21] Petroff and Stewart [22] and Seibert.[23]

Zinsser's concept of the relation of allergy and resistance is that the allergic state and increased resistance are parallel, and perhaps causally related, phenomena and that the substance on which allergy depends may possess protective functions differing, and based on another mechanism, from those possessed by antibodies.

Petroff and Stewart stated that it is now generally accepted that the resistance of animals to superinfection depends chiefly on the degree of the allergic state and that the absence of allergy, as demonstrated by the reaction to the intracutaneous tuberculin test, spells susceptibility to infection. They found by experiments that guinea-pigs sensitized with killed tubercle bacilli and later inoculated with living tubercle bacilli outlived normal guinea-pigs infected in like manner which were used as controls. The vaccinated pigs were allergic.

Seibert, on the other hand, from experiments on sensitization with tuberculin protein concluded that a high degree of hypersensitiveness to tuberculin protein confers no immunity or increased resistance to

20. Branch, A., and Cuff, J. R.: J. Infect. Dis. **47**:151, 1930.

21. Zinsser, H., and Mueller, J. H.: J. Exper. Med. **41**:159, 1925.

22. Petroff, S. A., and Stewart, F. W.: J. Immunol. **12**:97, 1926.

23. Seibert, F. B.: Proc. Soc. Exper. Biol. & Med. **30**:1274, 1933.

subsequent tuberculous infections. On the contrary, it seems to hasten and extend the lesion and to be associated with much more extensive necrosis and caseation than are noted in nonsensitized animals. It is evident from these experiments that Seibert considered allergy in tuberculosis not only unnecessary to resistance but actually harmful.

Since it has been shown that allergy has a harmful aspect and that resistance may exist without allergy, it became important in the present experiments to study allergy as related to vaccination with B C G in an attempt to determine whether an efficient method of vaccination could be developed in which allergy was not produced. In the animals vaccinated by the four methods described allergy was studied in respect to (*a*) frequency and degree, (*b*) time of appearance after vaccination, (*c*) duration, (*d*) comparative duration of allergy and resistance, (*e*)

TABLE 1.—*Frequency and Degree of Allergy in Rabbits Vaccinated by Four Methods*

No. of Rabbits	Method	Allergy						
		++++	+++	++	+	Total Number Positive Reactions	Total Number Negative Reactions	Percentage Positive Reactions
35	Subcutaneous injection of living organisms.....	4	5	6	7	22	13	63
28	Intravenous injection of living organisms.....	2	1	4	9	16	12	57
26	Subcutaneous injection of killed organisms.....	0	0	2	8	10	16	38
42	Intravenous injection of killed organisms.....	0	0	0	0	0	42	0

effects of existing allergy in animals subsequently vaccinated, (*f*) relation to antibodies, (*g*) relation to resistance and (*h*) relation to lesions.

(*a*) The frequency and degree of allergy resulting from vaccination by the four methods are shown in table 1. Allergy was greatest both in frequency and in degree in the animals vaccinated subcutaneously or intravenously with living organisms. The degree was much less in the animals vaccinated subcutaneously with heat-killed B C G. No allergy occurred in the animals vaccinated intravenously with heat-killed organisms.

From the standpoint of the danger of producing allergy by the process of vaccination, the method of administering the vaccine intravenously as heat-killed organisms proved to be the one of choice. The degree of allergy in the group of animals vaccinated subcutaneously with heat-killed organisms was slight, and as shown later in the experiments, such allergy soon disappeared.

(*b*) The time of appearance of allergy carries with it a good deal of significance from the point of view of the effect of allergy on a

second infection. In most discussions of primary and secondary pulmonary tuberculosis it is assumed that the first type develops in the absence of allergy while the second is influenced in its progress by allergy. Rothschild, Friedenwald and Bernstein in experimental tuberculosis in guinea-pigs found that allergy appeared as soon as seven days after an infection with human tubercle bacilli.

In my experiments the animals were regularly tested for allergy three weeks after the last injection of the vaccine. If allergy had not appeared by this time it was found by many repeated tests that it did not appear. In 100 consecutive animals in which positive reactions to the Mantoux test were obtained allergy was present at the latest in three weeks after the last injection. It regularly appeared three weeks after a single subcutaneous injection of living organisms, and in one series

TABLE 2.—*Duration of Allergy Resulting from Vaccination with BCG*

Number	Initial Degree of Allergy	Number of Months after Vaccination	Allergy, Degree
1	++++	3	++
2	++++	3	++
3	++++	3	0
4	+++	3	0
5	+++	3	0
6	+++	3	0
7	+++	1	0
8	++	2	0
9	++	1	0
10	++	1	0
11	+	1	0
12	+	1	0
13	+	1	0
14	+	1	0

tested allergy was present two weeks after the sensitizing injection. It may be said that allergy appears early in the development of lesions and that few if any tubercles observed are developed without the influence of allergy.

(*c*) The duration of allergy has a significant bearing from the standpoint of its effect on superinfection in the pathogenesis of tuberculosis. In table 2 is shown the duration of allergy in 14 vaccinated animals. It was noted that severe allergy, grades +++ and ++++, due to vaccination with B C G disappeared in about three months. A less degree tended to disappear in a shorter time. It is probable that allergy associated with vaccination with B C G disappears more rapidly than the allergy resulting from an infection with virulent bacilli, for lesions due to B C G tend to heal more rapidly and, as has been shown, allergy seems to be dependent on lesions.

(*d*) The comparative duration of allergy and resistance has been a question of considerable dispute. It has commonly been assumed that without allergy there is no resistance. If this were true, according

to the aforementioned findings, resistance due to vaccination could last but about three months. Willis,[24] in experimental tuberculosis in guinea-pigs, found the animals to be highly resistant to an inoculation of virulent bacilli after the allergy due to a preexisting infection with an organism of lower virulence had passed off.

In table 3 are shown the results obtained for 8 rabbits in which the duration of allergy and resistance was measured and compared. Three weeks after the last injection of the vaccine all 8 animals were allergic, from ++ to ++++. Just before the animals were given injections subcutaneously of 0.01 mg. of virulent bovine bacilli the allergy had disappeared entirely in 4 rabbits and remained +++ in 4. The time between the vaccination and the injection of virulent organisms

TABLE 3.—*Comparative Duration of Allergy and Resistance in Rabbits Vaccinated with BCG and Later Inoculated Subcutaneously with 0.01 Mg. of Virulent Bovine Tubercle Bacilli (Ravenel)*

		Allergy, Injection of Virulent Bacilli		Resistance, Degree of Tuberculosis Three Months after Injection of Virulent Bacilli	
Number	Initial	Before*	Three Months After	Lungs	Kidneys
1	++	0	0	0	0
2	+++	0	0	0	0
3	+++	0	0	0	0
4	+++	0	0	0	0
5	+++	+++	0	0	0
6	+++	+++	0	0	0
7	+++	+++	0	0	0
8	++++	+++	0	0	0

* Inoculation was made from two to four months after vaccination.

was from two to four months. Three months after the injection of virulent bacilli, the time at which the animals were killed for examination, all gave negative reactions to the Mantoux test. At this time none of the animals showed tuberculosis. Nonvaccinated animals used as controls had extensive generalized tuberculosis. From the observations in this experiment it was obvious that resistance can exist in the absence of allergy and may outlast allergy.

(*e*) The effects of existing allergy on animals when subsequently vaccinated have to be considered carefully in the practice of vaccinating with BCG, especially if the intravenous method of vaccination is used. Vaccination of persons against tuberculosis so far has been limited to nonallergic subjects. It has been suggested from my experiments that vaccination of allergic persons might greatly increase the degree of resistance. The question whether such vaccination could be done safely arose.

24. Willis, H. S.: Am. Rev. Tuberc. **17**:240, 1928.

In the discussion of toxic effects from vaccinating normal animals reference was made to the different results observed when animals already allergic were vaccinated intravenously, especially with large doses. The harmful results in such animals from the standpoint of toxic effects are shown in tables 4 and 5. Five rabbits were made allergic by giving each of them subcutaneously an injection of 1 mg. of living B C G (table 4). Three weeks later these animals were tested for allergy. The reactions of the first 4 animals were + + +, + + +, + + and +, respectively. The fifth animal showed no allergy. Each rabbit then received an injection intravenously of 4 mg. of heat-killed B C G. The first 3 animals collapsed after a few hours and died within twenty-four hours. The fourth became sick but survived. The fifth showed no ill effects from the injection. Mantoux tests showed that the fourth and fifth animals still gave reactions of + and —, respectively. Each animal was then given an intravenous injection of 3 mg. of heat-killed B C G. The fourth animal died within twenty-four hours, and the fifth remained well.

TABLE 4.—*Degree of Allergy Obtained by Injecting Heat-Killed BCG Intravenously into Animals Previously Given Subcutaneous Injections of 1 Mg. of Living BCG*

Animal	Amount of Killed BCG Injected, Mg.	Result	Reaction to Mantoux Test
1	4	Death in 24 hours	+++
2	4	Death in 24 hours	++
3	4	Death in 24 hours	+++
4	4	Ill	+
5	4	No change	0
4*	3	Death in 24 hours	+
5	3	No change	0

* Experiments on rabbits 4 and 5 were repeated.

TABLE 5.—*Allergic Reactions Obtained by Four Weekly Injections Intravenously of Heat-Killed BCG (0.25, 0.5, 0.5 and 0.5 Mg.) into Animals Previously Made Allergic by Injection of 4 Mg. of Living BCG Subcutaneously*

Number	Reaction to Mantoux Test			Condition of Animal
	Three Weeks after Subcutaneous Injection	Two Weeks after Last Intravenous Injection	Four Weeks after Last Intravenous Injection	
1	++++	0	+++	Well
2	+++	0	+	Well
3	+++	0	+	Well
4	+++	0	0	Well
5	+++	0	0	Well

Another experiment showed that with smaller doses allergic animals could be vaccinated intravenously without toxic results (table

5). In this experiment 5 rabbits were made allergic by a subcutaneous injection of 4 mg. of living B C G. Three weeks after this subcutaneous injection the animals were tested for allergy and found to give ++++, +++, +++, +++ and +++ reactions. They were then vaccinated intravenously with heat-killed B C G, first with 0.25 mg. and on the three following occasions with 0.5 mg. Two weeks after the last injection of the vaccine all the animals were well and completely desensitized, as indicated by a negative reaction to the Mantoux test. Four weeks after the last injection of the vaccine the animals were still well, but allergy had returned in the first 3 rabbits in grades, respectively, of +++, + and +. The results obtained in respect to toxic effects from vaccinating allergic animals with B C G showed that when the animals were given intravenous injections of large doses toxicity to the extent of producing death occurred. However, with small doses of heat-killed B C G administered intravenously, allergic animals could be vaccinated without toxic effects. Such animals

TABLE 6.—*Allergy and Humoral Antibodies in Animals Vaccinated with BCG*

Number	Allergy	Average Agglutination Titer	Average Complement-Fixation Titer
10	++ to +++	1:375	1:250
15	0	1:200	1:230

for a time at least were desensitized. The possibility of injury to visceral organs in vaccination of allergic animals is discussed in connection with the relation of lesions and methods of vaccination.

The results described in the experiments on allergic animals demonstrate a danger which should be taken into consideration in vaccinating children with B C G, especially if they give a positive reaction to the Mantoux test.

(*f*) The relation of allergy to antibodies is significant in vaccination with B C G, for, as is shown later, an elevation of antibodies in the serum tends to be correlated with the development of resistance. If there is a similar correlation between allergy and antibodies it might be assumed that allergy could be used as an indicator of resistance or that the absence of allergy indicated the absence of resistance. Rich and Lewis and Aronson [25] showed by experiments with tissue cultures that allergy existed in the tissues independent of antibodies in the circulating blood.

In table 6 are shown the average agglutination and complement-fixation titers for groups of allergic and nonallergic animals. In 10 animals

25. Aronson, J. D.: J. Exper. Med. **54**:387, 1931.

allergic reactions in degrees of from + + to + + + were produced. The average agglutination titer of these 10 animals was 1:375, and the average complement-fixation titer, 1:250. The second group of 15 animals had no allergy. The average agglutination titer of these 15 animals was 1:200, and the average complement-fixation titer, 1:230. The titers for the allergic animals were slightly higher than those for the nonallergic animals. This slight difference apparently was due not in any way to the allergy but to the fact that animals given injections of living organisms, especially intravenously, have higher antibody titers. The frequency with which such injections produce allergy is also high. The significant thing shown was that a relatively high antibody content could exist without associated allergy.

Another method of studying the relation of allergy to antibody content was by desensitizing allergic animals by means of intravenous injections of B C G (table 7). Five rabbits were made allergic by

TABLE 7.—*Relation of Allergy and Humoral Antibodies in Desensitized Animals Which Had Been Allergic to BCG* *

	Before Desensitization			After Desensitization		
Number	Reaction to Mantoux Test	Agglutination Titer	Complement-Fixation Titer	Reaction to Mantoux Test	Agglutination Titer	Complement-Fixation Titer
1	++	1:50	1:160	0	1:400	1:1,300
2	+++	1:50	1:160	0	1:400	1:1,500
3	+++	1:200	1:160	0	1:600	1:1,500
4	+++	1:200	1:160	0	1:800	1:1,500
5	+++	1:400	1:160	0	1:600	1:1,300

* Each of five control animals made allergic in a similar manner had a maximum complement-fixation titer of 1:160, which dropped in three weeks to from 1:0 to 1:80.

injecting subcutaneously 4 mg. of living B C G. Before desensitization of the animals by four weekly injections of heat-killed B C G the degree of allergy of 1 of these animals was + + and that of 4, + + +. The agglutination titers before desensitization were 1:50 and 1:400. The complement-fixation titers were all 1:160. After desensitization all animals gave a negative reaction to the Mantoux test. The agglutination titers rose in the process of desensitization to from 1:400 to 1:800 and the complement-fixation titers to from 1:1,300 to 1:1,500. In 5 sensitized animals used as controls the maximum complement-fixation titer was 1:160, which dropped in three weeks to from 0 to 1:80. This experiment showed that desensitization to the extent of producing complete absence of allergy could be brought about by intravenous vaccination and that the content of antibodies was increased in the process of desensitization.

Experimental results given in table 8 show that a high degree of allergy can be present in the absence of antibodies. Ten animals were

made allergic by one large subcutaneous injection of living B C G. Three weeks after the sensitizing injection the animals were tested for allergy. The first 8 animals had allergy of grade +++; the next 2, grade ++. The serums were tested for agglutinins at the same time. In none was there found a titer as high as 1:50.

What was found in regard to a proportionate or necessary relation between allergy and agglutinins and complement-fixing antibodies was also noted with respect to opsonins (table 9). It was shown in this

TABLE 8.—*Allergy and Agglutinins in Animals Three Weeks After Subcutaneous Injection in One Area of 4 Mg. of Living BCG*

Number	Reaction to Mantoux Test	Agglutination Titer
1	+++	0
2	+++	0
3	+++	0
4	+++	0
5	+++	0
6	+++	0
7	+++	0
8	+++	0
9	++	0
10	++	0

TABLE 9.—*Average Number of Bacilli (B C G) Phagocytosed by One Hundred Normal Mononuclear Leukocytes in One Hour in the Presence of Normal or Immune Serums (Dilution 1:75); Agglutination Titers of the Serums**

Number of Animals	Kind of Serum*	Number of Bacteria per 100 Leukocytes	Agglutination Titers
4	Normal	25	0
10	KS	58	1:70
10	LS	90	1:60
10	Defatted I	122	1:240
10	KI	130	1:265

* KS indicates serum immunized with heat-killed organisms injected subcutaneously; LS, that with living organisms injected subcutaneously; I, that with living organisms injected intravenously, and KI, that with heat-killed organisms injected intravenously.

experiment that greater phagocytosis took place with the serums of the animals which received intravenous injections of defatted or heat-killed B C G and that in none of these animals was allergy ever found.

From the observations in the four experiments just described it was concluded that no definite proportionate or necessary relation exists between the presence of bacterial allergy and the concentration of antibodies, such as agglutinins, complement-fixing antibodies and opsonins. From other experiments the same thing can be said in regard to lysins.

(*g*) The relation of allergy to resistance has been discussed in general in connection with the literature on allergy. The absence of correlation between the presence of allergy and antibodies, already mentioned,

suggested that allergy was not necessary to resistance. One necessarily should be certain that resistance can exist without allergy before attempting to vaccinate so as to get rid of the harmful effects of allergy.

Observations were made in the present experiments which gave information on this point. In table 10 the relation of allergy and resistance is shown. Forty vaccinated animals were selected, all of

TABLE 10.—*Allergy and Resistance in Animals Vaccinated with BCG and Later Inoculated Subcutaneously with 0.01 Mg. of Virulent Bovine Bacilli (Ravenel)*

Number	Allergy, Degree		Resistance as Indicated by Degree of Tuberculosis in	
	Intial	When Inoculated	Lungs	Kidneys
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	+	0
11	0	0	+	0
12	0	0	+	0
13	0	0	+	0
14	0	0	+	0
15	0	0	+	0
16	+	0	0	0
17	+	0	0	0
18	++	0	0	0
19	++	0	+	0
20	+++	0	0	0
21	+++	0	0	0
22	+++	0	0	0
23	+++	0	+	0
24	+	+	0	0
25	+	+	0	0
26	+	+	0	0
27	++	++	0	0
28	++	++	0	0
29	++	++	0	0
30	++	++	0	0
31	+++	+++	0	0
32	+++	+++	0	0
33	+++	+++	0	0
34	+++	+++	0	0
35	+++	+++	0	0
36	+++	+++	0	0
37	+++	+++	0	0
38	+++	+++	0	0
39	+++	+++	0	0
40	++++	+++	0	0

which were later inoculated subcutaneously with 0.01 mg. of virulent bovine tubercle bacilli. All showed either complete protection or only a slight amount of tuberculosis in the lungs, + on a basis of + + + +, ninety days after the injection of virulent bacilli. The degree of allergy was determined three weeks after the injection of the vaccine and again at the time of the injection of virulent organisms. Fifteen of the protected animals showed no allergy at any time, and 23 of the 40 animals were not allergic at the time of the injection of virulent

bacilli. Resistance existed equally well in the absence of allergy and in its presence. No obvious interdependence of allergy and resistance was noted. The findings in the experiments justified the attempt to develop resistance by a method of vaccination which produced adequate resistance without the harmful factor of allergy.

Lesions Due to Vaccination.—Another factor of safety to be considered in vaccinating with B C G is whether lesions are produced in the visceral organs during the course of vaccination. Many animals were vaccinated by the four methods already noted. In table 11 are recorded the observations in respect to lesions produced in internal organs of normal animals by each method. Microscopic examination revealed no lesions in the lungs, liver, spleen and kidneys after vaccination in any of the animals except in the group vaccinated intravenously

TABLE 11.—*Frequency and Degree of Lesions in Visceral Organs of Animals Vaccinated by the Four Methods*

No. of Animals	Methods	Lesions														
		Lungs					Liver					Spleen				
		++++	+++	++	+	0	++++	+++	++	+	0	++++	+++	++	+	0
10	Subcutaneously, living organisms	0	0	0	0	10	0	0	0	0	10	0	0	0	0	10
12	Intravenously, living organisms	1	3	1	4	3	0	4	0	0	8	0	4	0	0	8
10	Subcutaneously, heat-killed organisms	0	0	0	0	10	0	0	0	0	10	0	0	0	0	10
10	Intravenously, heat-killed organisms	0	0	0	0	10	0	0	0	0	10	0	0	0	0	10

with living organisms. In this group of 12 animals lesions of grades + to + + + occurred in the lungs of 9. Lesions of grade + + + were present in the liver of 4 animals. Four of the 12 animals had lesions in the spleen, all of grade + + +. It would appear, because of the frequency and degree of lesions resulting from this method, that it should be contraindicated as a means of vaccinating against tuberculosis. The other three methods in normal animals appeared to be safe.

Lesions of slight degree in some cases developed in the internal organs of allergic animals when vaccinated intravenously with heat-killed organisms in doses which did not cause lesions or toxic effects in normal animals. The lesions obtained by vaccinating allergic animals with small doses of heat-killed B C G are indicated in table 12. Six rabbits were made allergic by subcutaneous injection in one area in each animal of 4 mg. of living B C G. Three weeks after this sensitizing injection the animals were tested for allergy and found to give reactions of + + +, + + +, + + +, + +, + + and + +,

respectively. Each animal was then vaccinated with four injections of heat-killed B C G, the first dose being 0.25 mg. and each of the three remaining doses 0.5 mg. Three weeks after the last injection of the vaccine the animals were killed, and the organs were examined microscopically for the presence of lesions. Microscopic lesions of grades from + to + + were present in the lungs of all the animals and of grades from + + to + + + in the liver of 3. No lesions were noted in the spleen or kidneys of any of the 6 animals. The greatest frequency and degree of involvement tended to be in the animals having the greatest degree of allergy. In 10 nonallergic animals vaccinated in a similar

TABLE 12.—*Results Obtained by Vaccination of Allergic Animals*

Number	Involvement of Lungs	Involvement of Liver	Involvement of Spleen	Reaction to Mantoux Test
A. Allergic Reactions and Anatomic Changes Obtained by Four Weekly Injections Intravenously of Heat-Killed BCG (0.25, 0.5, 0.5 and 0.5 Mg.) into Animals Previously Made Allergic by Injection of 4 Mg. of Living BCG Subcutaneously				
1	++	+++	0	+++
2	++	++	0	+++
3	+	0	0	+++
4	++	+++	0	++
5	+	0	0	++
6	+	0	0	++
B. Allergy and Anatomic Changes Observed in Ten Animals Used as Controls Given Four Weekly Intravenous Injections of 1 Mg. of BCG				
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0

manner, except that 1 mg. of B C G was used in each injection, no lesions were detected in any of the organs. The experiments demonstrated an increased susceptibility to the development of lesions in allergic animals when given intravenous injections of heat-killed B C G. Repeated subcutaneous injections into many allergic animals always failed to produce lesions in the visceral organs. The increased susceptibility to lesions (though nonprogressive) noted in the allergic animals should be considered a dangerous factor in the vaccination of allergic persons. Further experiments are in progress to see whether by regulating the amount of vaccine given intravenously at each injection the development of lesions in the visceral organs can be prevented. The importance of the possibility of vaccinating allergic animals intravenously is obvious, for by this method of vaccination resistance in such animals is increased and desensitization is produced.

The following conclusions in respect to safety in the use of the four methods of vaccination with B C G can be stated: No mechanical injuries result from any of the methods. Immediate toxic effects do not occur. Delayed toxic effects occur only in allergic animals when vaccinated intravenously. Allergy occurred in greater degree and frequency in animals vaccinated with living organisms, subcutaneously or intravenously. Allergy was slight in animals vaccinated subcutaneously with heat-killed organisms. It did not occur at all in the animals given intravenous injections of heat-killed B C G. Allergy occurred early and soon passed off. It disappeared before resistance and bore no proportionate or causal relation to antibodies or resistance. In normal animals lesions (nonprogressive) in visceral organs were produced only by injecting living organisms intravenously, but in the allergic animals intravenous injections of heat-killed organisms produced a few lesions. Allergy was shown to have a harmful aspect and to be unnecessary for resistance. Except for the presence of allergy all the methods of vaccinating normal animals should be considered safe but the one in which living organisms are injected intravenously. The method in which heat-killed organisms were injected intravenously into normal animals was free from all the dangerous factors studied. Further experiments are needed concerning the safety of vaccinating allergic animals or persons, especially if the intravenous method of administering the vaccine is used.

RESISTANCE

Resistance is the chief factor to be considered in the use of B C G as a vaccine against tuberculosis, for unless a fair degree of increased resistance is developed vaccination, of course, is useless. Calmette and others have vaccinated a large number of children orally with B C G. Heimbeck's work demonstrated a degree of efficiency of B C G vaccine in reducing the number of cases of clinical tuberculosis developing among nurses exposed to open tuberculosis in the wards. Heimbeck vaccinated by injecting the living organisms subcutaneously. Park and his associates also vaccinated a large number of children subcutaneously with living B C G.

Birkhaug found that guinea-pigs vaccinated subcutaneously with living B C G lived longer than normal guinea-pigs inoculated with an equal amount of virulent human tubercle bacilli. Park and King [26] also reported that increased resistance was obtained by vaccinating animals with B C G against infections with virulent tubercle bacilli.

Resistance to tuberculous infection would necessarily have to be species-specific. Because of this fact I tried in my experiments to develop resistance to a virulent bovine strain (Ravenel) and to the

26. Park, W. H., and King, M. J.: Am. J. Pub. Health **19**:179, 1929.

human strain (H 37). Rabbits were used in experiments with the bovine strain and guinea-pigs in those with the human strain. The evidences of resistance were (1) elevation of the number of antibodies in the serum of the vaccinated animals and (2) protection of the animal against lethal inoculations of virulent bacilli. The antibodies studied in relation to resistance were agglutinins, complement-fixing antibodies, opsonins and lysins.

TABLE 13.—*Average Agglutination Titers in Rabbits Vaccinated by Four Methods*

No. of Animals	Method	Agglutination Titers
22	Subcutaneous injection of living organisms	1:300
10	Intravenous injection of living organisms	1:700
38	Subcutaneous injection of killed organisms	1:100
53	Intravenous injection of killed organisms	1:300

TABLE 14.—*Degree of Tuberculosis in Normal and Vaccinated Rabbits Ninety Days After a Subcutaneous Inoculation of 0.01 Mg. of Virulent Bovine Tubercle Bacilli; Agglutination and Complement-Fixation Titers*

	Normal Animals				Vaccinated Animals			
No.	Tuberculosis in Lungs	Tuberculosis in Kidneys	Agglutination Titer	Complement-Fixation Titer	Tuberculosis in Lungs	Tuberculosis in Kidneys	Agglutination Titer	Complement-Fixation Titer
1	+	0	0	0	0	0	1:50	1:160
2	++	0	0	0	0	0	1:50	1:160
3	+++	0	0	0	0	0	1:50	1:160
4	+++	+	0	0	0	0	1:50	1:160
5	+++	+	0	0	0	0	1:50	1:160
6	+++	+	0	0	0	0	1:200	1:324
7	+++	+++	0	0	0	0	1:400	1:324
8	+++	+++	0	0	0	0	1:800	1:648
9	++++	+++	0	0	0	0	1:800	1:648
10	++++	+++	0	0	0	0	1:3,200	1:648
11	++++	++++	0	0	0	0	1:400	1:1,295
12	++++	++++	0	0	0	0	1:600	1:1,295
13	++++	++++	0	0	0	0	1:800	1:2,500
14	++++	++++	0	0	+	0	1:50	1:80
15	.....	.....	.....	.....	+	0	1:50	1:160
16	.....	.....	.....	.....	+	0	1:100	1:160
17	.....	.....	.....	.....	+	0	1:200	1:160
18	.....	.....	.....	.....	+	0	1:800	1:160
19	.....	.....	.....	.....	++	+	<1:50	1:324
20	.....	.....	.....	.....	+++	0	1:200	1:648

The average agglutination titers for animals vaccinated by the four methods are recorded in table 13. The average maximum titer for the 22 animals vaccinated subcutaneously with living organisms was 1:300; for the 10 animals vaccinated intravenously with living organisms, 1:700; for the 38 animals vaccinated subcutaneously with heat-killed organisms, 1:100, and for the 53 animals vaccinated intravenously with heat-killed organisms, 1:300.

In table 14 is shown a comparison of the degree of tuberculosis in normal and in vaccinated rabbits ninety days after inoculating them subcutaneously with 0.01 mg. of the virulent bovine strain. The agglu-

tination and complement-fixation titers before the injection of virulent organisms are also given in each case. In none of the 14 normal rabbits were antibodies detected. Tuberculosis of degrees from + to ++++ was present in the lungs of all the rabbits. In only 2 of the animals was the degree less than +++. Tuberculosis was present in the kidneys of all but 3 rabbits in grades of from + to ++++.

The vaccinated animals in the main showed a decided elevation of antibody titer. Complete protection was noted in 13 of the 20 animals and, as compared with the normal animals used as controls, also in the 7 remaining rabbits. The lowest dilution tested for agglutinins was 1:50. All but 1 of the animals had a titer higher than this. The lowest dilution tested for complement-fixing antibodies was 1:30. All protected animals had a titer higher than this. As a rule, the content of antibodies in the serum was decidedly elevated in the vaccinated protected animals.

The average number of B C G bacilli phagocytosed by 100 normal mononuclear leukocytes in one hour at 37 C in the presence of normal or immune serums is noted in table 9. The degree of phagocytosis is compared with the agglutination titer. The average number of bacteria phagocytosed was greater for the immune than for the normal animals. The animals vaccinated with defatted or heat-killed organisms showed the highest percentages of phagocytosis and also the highest agglutination titers. The degree of phagocytosis was correlated with the height of the agglutination titer.

The lytic action referred to in this paper is that indicated by the disappearance of organisms so that they are not shown either by acid-fast stains or by ordinary methods of staining. It seems to be necessary for the organisms to be phagocytosed before being lysed. The technic for determining the lytic power of a serum in the presence of mononuclear cells will be reported in another paper.

The phagocytic power and lytic action of serums on B C G when mixed with mononuclear leukocytes are recorded in table 15. The lytic action greatly increased as the agglutination titer rose. The number of the nonlysed bacilli which were phagocytosed also greatly increased with the increase in the titer of the agglutinins.

The concentration of antibodies was increased by all the methods of vaccination. The findings in respect to phagocytosis and lysis suggested that the degree of destruction of organisms (lysis) was correlated with the degree of concentration of antibodies in the serums.

The final and surest proof of resistance was protection observed in animals against inoculation with lethal doses. In the rabbits the degree of resistance was measured by the amount of tuberculosis present in the lungs and kidneys from forty-five to ninety days after subcutaneous injection of 0.01 mg. of virulent bovine tubercle bacilli. These organs

were the usual sites of the first development of tuberculosis in rabbits. The amount of tuberculosis seen in the spleen, liver and kidneys of guinea-pigs was used as a standard to measure the protection in these

TABLE 15.—*Phagocytic and Lytic Action of Normal Mononuclear Leukocytes on BCG When a Mixture of Equal Volumes of Leukocytes, BCG and Normal or Immune Serum (Dilution 1:75) is Incubated with Constant Mixing at 37 C. for One Hour; Agglutination Titers of Serums*

Serums		Agglutination Titers	Average Percentage of Bacteria Remaining Visible after Incubation		
Number	Kind		Total	Inside Leukocytes	Outside Leukocytes
4	Normal	0	100	7	93
9	Immune	1:50 or less	71	19	81
4	Immune	1:100	45	25	75
6	Immune	1:200	40	19	81
2	Immune	1:400	20	31	69
4	Immune	1:800	15	86	14
2	Immune	1:1,600	8	80	20
2	Immune	1:3,200	8	92	8
3	Immune	1:6,400	5	95	5

TABLE 16.—*Degree of Tuberculosis in Normal and Vaccinated Rabbits from Forty-Five to Ninety Days After Subcutaneous Inoculation with 0.01 Mg. of Virulent Bovine Bacilli (Ravenel)*

			Rabbits Vaccinated with					
	Normal Rabbits		Living BCG Subcutaneously		Killed BCG Subcutaneously		Killed BCG Intravenously	
No.	Lungs	Kidneys	Lungs	Kidneys	Lungs	Kidneys	Lungs	Kidneys
1	++	0	0	0	0	0	0	0
2	+++	0	0	0	0	0	0	0
3	+++	0	0	0	0	0	0	0
4	+++	+	0	0	0	0	0	0
5	+++	+	0	0	0	0	0	0
6	+++	+	0	0	0	0	0	0
7	+++	++	0	0	0	0	0	0
8	+++	+++	0	0	0	0	0	0
9	+++	+++	0	0	+	0	+	0
10	++++	+++	0	0	+	0	+	0
11	++++	+++	0	0	+	0	+	0
12	++++	+++	+	0	+	0	++	+
13	++++	++++	+	0	+	0	++	+
14	++++	++++	+	0	+	0	++	+
15	++++	++++	..	..	++	0	++	++
16	++++	++++	..	..	+++	0	+++	+
17	..	..	..	..	+++	+	+++	++
18	..	..	..	..	++++	++++	+++	+++
19	..	..	..	..	..	..	++++	+++
20	..	..	..	..	..	..	++++	+++

animals. It was noted that, as a rule, tuberculosis in guinea-pigs developed in these organs in the order of frequency named.

The degree of tuberculosis in normal rabbits and in rabbits vaccinated by three of the aforementioned methods from forty-five to ninety days after a subcutaneous inoculation of 0.01 mg. of the bovine strain is shown in table 16. On account of the large amount of allergy and the

frequency and degree of lesions in animals vaccinated intravenously with living organisms this method was dropped early from subsequent experiments. A marked degree of resistance was noted in rabbits vaccinated by all three methods. It is doubtful from these experiments whether, from the standpoint of resistance, one method can be said to be superior to another.

Injection into the lung through the trachea of 1 mg. of living BCG was made in 10 rabbits (table 17). Later, these 10 and 4 normal animals were inoculated subcutaneously with 0.01 mg. of virulent bovine tubercle bacilli. The injections were made on the same day from the same suspension. All animals were killed forty-five days after the injection of virulent bacilli. The normal animals showed extensive tuberculosis.

TABLE 17.—*Degree of Tuberculosis Forty-Five Days After a Subcutaneous Inoculation of 0.01 Mg. of Virulent Bovine Tubercle Bacilli (Ravenel) in Normal Rabbits and in Rabbits Which Had Previously Been Given Injections in the Lung of 1 Mg. of Living BCG*

	Normal Rabbits		Rabbits Receiving Injections of BCG in the Lung	
Number	Lungs	Kidneys	Lungs	Kidneys
1	+++	0	0	0
2	+++	++	0	0
3	++++	+++	0	0
4	++++	++++	0	0
5	..	..	0	0
6	..	..	0	0
7	..	..	0	0
8	..	..	0	0
9	..	..	+	0
10	..	..	+	0

The animals previously given injections of living BCG into the lung were all free from tuberculosis, except 2 which showed a + degree in the lung only. Marked resistance was noted in the animals previously vaccinated with BCG. In several of these animals a nodule or two were present in the lung at the point of injection of the BCG. Eighty per cent of such animals were allergic as a result of the injection. This experiment gave useful information in regard to the possible advantage of a healed Ghon tubercle in the lung.

The protection given to guinea-pigs against infection with the human strain (H 37) by vaccinating the animals with living BCG is indicated in the observations recorded in tables 18 and 19. In the first group (table 18) there were 18 normal and 15 vaccinated guinea-pigs. They died or were killed after inoculation with H 37 at periods ranging from thirty-two to ninety-six days. They were then examined for gross and microscopic evidence of tuberculosis. The degree of tuberculosis was estimated from the amount present in the spleen, liver and lungs. Tuber-

TABLE 18.—*Degree of Tuberculosis and Allergy (Due to Vaccination) in Normal and Vaccinated Guinea-Pigs from Thirty-Two to Ninety-Six Days After a Subcutaneous Inoculation of 0.05 Mg. of H 37*

Number	Normal Animals		Vaccinated Animals	
	Tuberculosis	Allergy	Tuberculosis	Allergy
1	++++	0	0	++++
2	++++	0	0	++
3	++++	0	0	++
4	++++	0	0	++
5	++++	0	0	++
6	++++	0	0	++
7	++++	0	0	++
8	+++	0	0	++
9	+++	0	0	++
10	+++	0	0	++
11	+++	0	0	+++
12	+++	0	0	++
13	+++	0	+++	++
14	+++	0	++++	++
15	+++	0	++++	+++
16	+++	0		
17	+++	0		
18	+	0		

TABLE 19.—*Degree of Tuberculosis in the Organs of Normal and Vaccinated Guinea-Pigs After a Subcutaneous Inoculation of 0.05 Mg. of H 37*

Number of Days after Inoculation	Normal Animals				Vaccinated Animals			
	Number	Spleen	Liver	Lungs	Number	Spleen	Liver	Lungs
27	1	++	0	0	2	0	0	0
						0	0	0
28-29	2	+	0	0	2	0	0	0
		+++	++++	0		0	0	0
30	2	+	0	0	2	0	0	0
		+++	+++	0		0	0	0
33-36	3	+++	+++	0	1	0	0	0
		++++	++++	0				
		++++	++	+++				
37	4	++	0	0	1	+	0	0
		+++	+++	0				
		++++	0	+++				
		++++	+++	+++				
38-39	1	++++	++++	++	3	0	0	0
						0	0	0
						0	0	0
40	1	++++	++++	+++	2	0	0	0
						0	0	0
42-45	1	++++	++++	+++	2	0	0	0
						+	0	0
46-50	2	+++	+++	+	1	+++	0	0
		++++	++++	+++				
58	1	++++	++++	+++	1	0	0	0
60	11	0	0	+	12	0	0	0
		+	++	0		0	0	0
		0	+	++		0	0	0
		0	+	+++		0	0	0
		+++	0	0		0	0	0
		+++	++++	+++		0	0	0
		+++	++++	++++		0	0	0
		++++	++++	0		+++	0	0
		++++	++++	++		++	0	+
		++++	++++	++++		+++	0	0
		++++	++++	++++		++++	0	+
						++++	++++	++++

culosis was observed in all the normal guinea-pigs; grade $++++$ was noted in 7, grade $+++$ in 10 and grade $+$ in 1. Allergy was not present before the injection of virulent organisms in any of the guinea-pigs. Of the 15 vaccinated animals tuberculosis was absent in 12. It was present in the 3 remaining animals in grades from $+++$ to $++++$. Allergy resulting from the vaccination was present in grades from $++$ to $+++$ at the time of injection with H 37.

In the second group (table 19) 29 normal and 29 vaccinated guinea-pigs were inoculated subcutaneously with 0.05 mg. of H 37 in an area about half-way between the axillary and the inguinal region. The amount of tuberculosis in the spleen, liver and lungs of the animals of each group is shown for the various periods at which the animals died or were killed. Twenty-seven days is the shortest time in which tuberculosis developed in the nonvaccinated animals.

When animals from the normal or the vaccinated group died a corresponding number from the other groups were killed. Sixty days after the inoculation with H 37 all the animals still living were killed. All animals were examined grossly and microscopically for the presence and degree of tuberculosis. These vaccinated guinea-pigs showed a marked degree of resistance to the human tubercle bacilli.

COMMENT

A series of experiments is described concerned with immune reactions in experimental tuberculosis. The purpose of the study was to determine an experimental basis for the safest and most efficient method of using the Calmette-Guérin bacillus (B C G) in developing resistance to bovine and human tuberculosis. Another consideration in the experiments was the relation of the reactions included in immunity to the pathogenesis of tuberculosis.

In conformity with the course pursued in previous experiments on vaccination with streptococci, the B C G vaccine was administered as living organisms subcutaneously and intravenously and as heat-killed organisms in the same ways. The vaccine so administered was studied in respect, first, to safety and, second, to resistance. No mechanical injuries, such as bacterial emboli or any other kind of tissue injury, were at any time observed from vaccinating many animals by each of the four methods.

No toxic results, either immediate or delayed, were noted in the normal animals after vaccination. With large doses given intravenously to animals already allergic, extreme collapse and death within twenty-four hours took place, but ordinary doses were not accompanied by toxic effects in the allergic animals. Subcutaneous injections of the vaccine into allergic animals had no ill effects. In allergic animals vaccinated

with relatively small doses intravenously, small, nonprogressive tubercles frequently developed in the lungs, liver and spleen. It would seem, until further observations and experiments are made, that vaccination of persons should be limited to those not having a positive reaction to the cutaneous tuberculin test. However, it would be an advantage if it is found that allergic animals can safely be vaccinated intravenously, for in the process of vaccinating the allergic animals they are desensitized, at least for a time, and resistance is increased.

Allergy was studied as a factor in safety and also in respect to its influence in the pathogenesis of tuberculosis. The frequency and degree of allergy were greater after vaccination by methods in which the living organisms were injected, but even by these methods allergy did not develop in all animals. A relatively small amount of allergy was produced in the animals given subcutaneous injections of heat-killed organisms. No animals vaccinated intravenously with heat-killed organisms ever became allergic.

It was found that severe allergy following vaccination disappeared in about three months and less severe allergy in less time, usually in about one month. This disappearance of allergy was probably more rapid than in persons in whom allergy is so frequently due to arrested active lesions. But it seems probable that any allergy, especially of the smaller degrees, which develops in the course of vaccination with B C G should not be looked on as a serious handicap.

Allergy in association with vaccination and probably with the development of a tubercle appears much earlier than is generally thought. It has been found to appear in from one to three weeks. In these experiments if allergy had not appeared in three weeks after the last injection of the vaccine it was found that it would not occur. The fact that allergy appears so early has to be considered in the consideration of the reaction of primary and secondary tuberculosis. It would seem that tubercles which are not influenced by the allergic state are seldom seen.

The experiments showed that allergy definitely tended to disappear before resistance. This should be remembered if a positive result in a test for allergy is looked on as an indicator of immunity. It is also a fact to keep in mind in coming to a conclusion on the much disputed question of whether allergy is a necessary part of the immune (resistant) state.

The experiments seemed to show that there is no proportionate or necessary relation between allergy and the immune bodies, agglutinins, complement-fixing antibodies, opsonins and lysins. In this respect, allergy in tuberculosis seems to differ from the Arthus phenomenon. A high antibody content could be developed in animals without allergy; on the other hand, a high degree of allergy could occur without measurable

antibodies. Allergic animals were desensitized so that they failed to give a positive Mantoux reaction, and in the process of desensitization the number of antibodies was greatly increased.

It was found that allergy never occurred in animals in which lesions could not be found. This supported the dictum of Krause:[27] "No tubercles, no allergy." In vaccinating with B C G or with other preparations in order to avoid the development of allergy, some method should be used which will not cause lesions.

The significance of allergy in the immune state has caused a great deal of debate. These experiments supported the conclusions of those investigators who contend that allergy is not a necessary factor in resistance. This was shown by three methods: (1) by vaccinating animals so as not to produce any initial allergy; (2) by waiting for the allergy to disappear before inoculating an animal with a virulent strain of bacillus, and (3) by desensitizing allergic animals by intravenous injections of heat-killed B C G and then giving an injection of a virulent strain and observing the resistance in the absence of allergy. Allergy has dangerous aspects, such as allergic shock and increased susceptibility to the development of lesions, but when it is taken into consideration that allergy tends to occur in a relatively small percentage of cases and in small degrees or not at all by adequate methods of vaccination, it is doubtful whether such an amount of allergy should be looked on as a very serious condition.

Only one of the four methods of vaccination in normal animals resulted in producing small, nonprogressive tubercles in the lungs, liver and spleen. This was the method in which living organisms were injected intravenously. Living organisms injected subcutaneously, even into allergic animals, produced no lesions in the visceral organs. Small lesions were produced by injecting heat-killed organisms intravenously into animals already allergic.

In general it can be said, as far as safety in the process of vaccination with B C G is concerned, that all methods are safe except the one in which the living organisms are injected intravenously. Even by this method progressive tuberculosis does not occur, but too many nonprogressive tubercles develop in the lungs, liver and spleen after the administration of the vaccine to permit one to consider it a safe method. The observations in the experiments did not justify the injection of living B C G vaccine intravenously.

Evidence of resistance due to vaccination against the virulent bovine strain of the tubercle bacillus in rabbits and against the virulent human strain in guinea-pigs was shown in two ways: (1) by a correlated increase in the titers of agglutinins, complement-fixing antibodies,

27. Krause, A. K.: Tr. Nat. A. Prev. Tuberc. **17**:348, 1921.

opsonins and lysins, and (2) by actually preventing or greatly retarding the development of tuberculosis in rabbits and in guinea-pigs after inoculations with virulent strains of tubercle bacilli.

CONCLUSIONS

Vaccination of nonallergic animals by any of the four methods described is not followed by mechanical injuries or by immediate or delayed toxic results. With large doses of the vaccine administered intravenously to allergic animals toxic effects may follow, but with moderate doses no delayed toxicity is noted.

Allergy is greatest in frequency and degree in animals vaccinated subcutaneously with living B C G, next greatest in animals vaccinated intravenously with living B C G and least in animals vaccinated subcutaneously with heat-killed B C G; but it does not occur at all in animals vaccinated intravenously with heat-killed B C G.

Allergy appears at the latest in from two to three weeks. It tends to disappear in from one to three months, depending on the degree.

Allergy never develops in the absence of lesions. The susceptibility to the development of lesions is increased by existing allergy. Allergy disappears more rapidly than coexisting resistance. There appears to be no proportionate or necessary correlation between the presence of allergy and the existence or concentration of antibodies in the serum. Allergy bears no proportionate or necessary relation to resistance. Definite resistance may be obtained to infection with bovine or human strains by vaccinating with B C G. The degree of resistance tends to be correlated with concentration of the antibodies in the blood.

Resistance coexistent with allergy more than compensates for the harmful effects of allergy.

The experiments suggest the possibility of safe and efficient vaccination of persons with B C G against ordinary degrees of infection with bovine or human strains of tubercle bacilli.

ACUTE ULCERATIVE ESOPHAGITIS

A PATHOLOGIC AND CLINICAL STUDY OF EIGHTY-TWO CASES OBSERVED AT NECROPSY

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Whether certain changes in the esophagus which are found at postmortem examination take place before or after death has been a subject of controversy since Hunter,[1] in 1786, reported his observations on self-digestion of the esophagus. Pringle, Stewart and Teacher [2] reviewed the whole subject in 1921. None of the many articles which have been written in the interim will be mentioned. Moutier,[3] in 1921, reported 3 cases of acute postoperative esophagitis. Subsequently Henke and Lubarsch [4] and Bell [5] have contributed to the subject.

MATERIAL STUDIED

The material for this study consisted of pathologic specimens and clinical data gathered at the Mayo Clinic over a period of about seven years. Among 6,000 fresh and preserved esophageal specimens which were obtained at necropsy, 82 instances of the condition which is designated here as acute ulcerative esophagitis were found, an incidence of 0.013 per cent. Because of the direct information regarding these patients, it was possible to attempt correlation between the clinical and the pathologic observations.

PATHOLOGIC STUDY

In all of the cases, the esophagus was found to be dilated at necropsy, especially in the lower third, and to contain gastric contents. The flow between the stomach and the esophagus was uninterrupted,

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1. Hunter, John: The Works of John Hunter, London, Longman, Rees, Orme, Brown, Green and Longman, 1835, vol. 1.

2. Pringle, J. H.; Stewart, L. T., and Teacher, J. H.: J. Path. & Bact. **24:** 396, 1921.

3. Moutier, F.: J. A. M. A. **76:**1536, 1921.

4. Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 6, pt. 1.

5. Bell, E. T.: A Text-Book of Pathology, Philadelphia, Lea & Febiger, 1930, p. 459.

as the supposed cardiac sphincteric action was not present. Ulcerations were not found in any case in which the esophagus was firm with the normal, folding rugae present. In specimens which had been subjected to the stretching, dilating force of the contained fluid, these folding rugae were obliterated.

The wall of the esophagus usually was pliable and thinned, and in some cases the color of the fluid which it contained could easily be made out. The mediastinum was involved in only 3 cases. In 2 of these cases perforation had taken place into the pleura, and in the other case purulent mediastinitis apparently had followed extensive ulceration of the esophagus and was a contributing cause of death. The mediastinitis was partially localized around the lower third of the esophagus.

The changes which were seen when the esophagus was opened usually were limited to the entire circumference of the lower third or half of the viscus. In only 2 cases was almost the entire esophagus involved.

The type of lesions, from the gross standpoint, varied greatly and may be described as follows: (1) phlegmonous and pseudomembranous ulcerations; (2) irregular superficial coalescing ulceration with hemorrhage or with bile staining; (3) linear or longitudinal ulceration with hemorrhage or black eschar; (4) simple erosions, petechiae-like lesions with congestion, and (5) perforating ulcerations, which varied in size and shape.

The extent of the ulcerative change varied somewhat. Occasionally the ulceration was moderate, but in most cases it was diffuse and widespread, leaving only a small amount of mucosa, in island-like formation. The residual mucosa appeared somewhat softened and could be rubbed off by pressure of the finger, leaving the submucosa and muscularis.

The gross appearance usually predicated the type of lesion that would be seen microscopically, but this was not uniformly true, for the autolysis incident to postmortem changes altered the true picture and minimized the inflammatory nature of the lesion. Microscopically, the ulcerations could be grouped into four main types: (1) pseudomembranous ulceration; (2) simple ulceration with slight or marked inflammatory change; (3) hemorrhagic ulceration, and (4) phlegmon of the entire wall, similar to that which accompanies perforation.

Pseudomembranous ulceration was characterized microscopically by more or less replacement of the normal squamous mucosal layer with one which consisted of cellular and fibrinous débris, which might be stained with bile. Beneath this layer there was found evidence of infiltration of the remaining submucosa by leukocytes, mostly polymorphonuclears. The degree of infiltration might be slight or so extensive that the entire submucosa and muscularis were involved. The

blood vessels, if present, usually were not engorged, and there was little, if any, hemorrhagic infiltration of the tissues. This type of ulceration rarely bled.

Simple ulceration was characterized by denuding the tissue of the squamous layer of epithelium, without replacement (fig. 1*A*). The submucosa was infiltrated with an inflammatory cellular exudate. At times, this was mild, but it might be severe and involve all the layers down to the muscularis. Here and there, small masses of degenerating erythrocytes might be seen. In cases in which there was severe infiltration, there were masses of necrosis in the submucosa. The nuclei of the normal tissue stained poorly.

Hemorrhagic ulceration (fig. 1*B* and *C*) represented the type that accounted for most of the bleeding, especially of the profuse type. The layer of squamous epithelium was lost. In the submucosa, the vessels were dilated and engorged, and erythrocytes infiltrated the tissues. Edema of the tissues usually was marked, and leukocytic infiltration varied from slight to moderate, but was never extensive. Occasionally, eosinophils were found to be numerous.

It was not difficult to understand the ease with which hemorrhage had been induced in these cases, when it was seen that the vessels were situated close under the squamous layer, especially if they were dilated, and that a slight inflammatory change would erode the walls of these vessels. Ulcerations might be responsible for the supposed spontaneous rupture of esophageal varices in cases of hepatic cirrhosis.

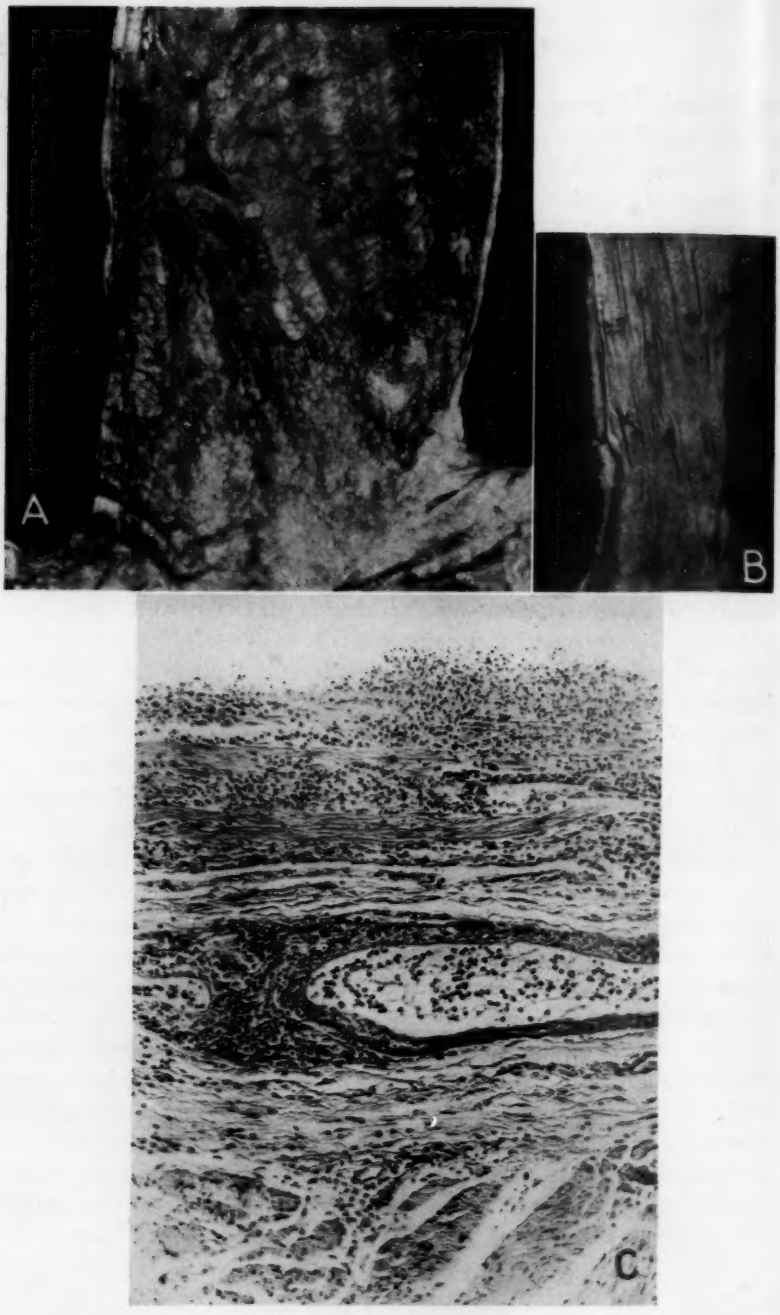
In phlegmonous ulcerations, which are diffuse types of ulceration, the normal constituents of the tissue were absent, and only leukocytic infiltration, in degenerating, necrotic, cellular débris, remained. The entire wall was thickly infiltrated by leukocytes for a considerable distance from the perforation, and there was gradual transition to the normal esophageal tissues.

The mucosa of the stomach was examined in a number of the cases in which there were severe changes in the esophagus, but inflammatory changes were not found. There was, however, slight digestion of the rugae, for they had lost some of their natural contour. This proves that there is some type of digestion which follows death, but this can be distinguished from antemortem conditions by the presence of inflammatory reaction in the latter.

CLINICAL STUDY

Pringle and Teacher [6] were the first to recognize the clinical importance of this condition. Their cases were of postoperative hematemesis of esophageal origin. The cases included in the present report were

6. Pringle, J. H., and Teacher, J. H.: Brit. J. Surg. **6**:523, 1919.



A, photomicrograph of multiple small hemorrhagic ulcers with many linear superficial ulcerations in the lower third of the esophagus; × 120. *B*, numerous deep and superficial ulcerations of the lower third of the esophagus. The deep ulcerations are hemorrhagic. *C*, hemorrhagic ulceration of the esophagus. The squamous layer is no longer present. There is a leukocytic infiltration throughout the submucosa, extending into the muscularis, and there is some edema of the tissues.

grouped and studied from the following standpoints: (1) primary cause of death; (2) age; (3) sex; (4) whether or not vomiting occurred, and if so, whether or not blood was present in the vomitus; (5) trauma from passage of a stomach tube; (6) symptoms referable to the esophagus, and (7) miscellaneous symptoms.

Primary Cause of Death.—Fifty-five deaths in this series followed operations. The remaining 25 patients who died were treated medically. The surgical cases were grouped as to procedures. In 13 cases, operation was performed on the colon; in 12, on the genito-urinary tract; in 8, on the stomach; in 13, on the gallbladder, bile ducts and pancreas; in 4, on the head; in 2, on the neck, including the thyroid gland; and in 10, on miscellaneous organs.

This grouping, because operations on the head are relatively infrequent in a surgical practice, suggests that esophagitis is slightly more likely to follow such operations than it is to follow others. The literature tends to bear this out. However, this is only suggestive. The presence of esophagitis in association with lesions of the brain proves that the prevalent surgical complication of general peritonitis is not an essential factor in the etiology of esophagitis. However, it might be said that all the operations were rather difficult and extensive, and occasionally, the patients constituted poor surgical risks.

The length of time between operation and death did not seem to be of significance, for the esophagitis appeared from one to fifteen days after operation, and in twenty cases it appeared later than this. Its severity also bore no relation to the number of days that had elapsed since the operation. High fever was a common clinical manifestation.

In the cases in which medical treatment was employed, acute ulceration of the esophagus occurred as follows: in 13 cases of sepsis or infection; in 6 cases of heart disease, and in 1 case each of tuberculosis, diabetes, uremia, fracture of the ribs with hemorrhage, leukemia and carcinoma of the pancreas. All these conditions were debilitating, and the patients died after prolonged illness, except a patient who had coronary disease and died twenty-four hours after an infarction. There were no sudden deaths in this group.

That the condition should be present in 6 cases of chronic ulcerative colitis seems to be more than coincidental and might suggest the possibility of susceptibility to ulceration. On the other hand, most of the patients were severely debilitated and 5 of them had had operations on the colon.

In a few cases mercurochrome had been administered intravenously because of sepsis and, in addition to ulceration of the esophagus, the rather typical ulcerations of the colon were present. Ulceration of the esophagus, however, occurred in too small a number of cases in which

mercurochrome was injected intravenously to warrant drawing any conclusion.

Age.—The age of the patients varied from 8 months to 78 years, and although it is difficult to judge its significance, because of numerous variables, it is not felt to be of any significance as an etiologic factor. The patients were distributed rather evenly in the adult periods. Three patients were between 8 months and 9 years of age; 2 were between 10 and 19 years; 10 were between 20 and 29 years; 12 were between 30 and 39 years; 13 were between 40 and 49 years; 18 were between 50 and 59 years; 15 were between 60 and 69 years; 8 were between 70 and 79 years of age, respectively, and 1 patient was aged 82 years.

Sex.—Twenty-four of the patients were females and 58 were males. However, males outnumbered females in the general registration at the clinic, and consequently more males than females were subjected to operation. The number of deaths from surgical procedures which involved the genito-urinary tract was also high and chiefly affected males. I feel that the factor of sex is of no significance.

Vomiting.—Gross vomiting occurred in 59 cases, and in the remaining 23 cases, little, if any, vomiting was observed. The amount of vomitus was extremely variable. In 18 cases, it was considerable, and in the remainder it was only slight or moderate. Vomiting had been present from one to fourteen days in most cases, and in only 4 had it persisted as long as six weeks, and then only at intervals.

Blood was prominent in the vomitus in 15 of the 59 cases in which vomiting occurred. It was difficult or impossible to ascertain the amount of blood vomited, for considerable gastric content, which could not easily be separated, was associated with the vomitus. Amounts of gastric content which contained blood varied from 50 to 2,000 cc. The vomitus was usually described as having had the appearance of coffee grounds, but in 1 case it was bright red. In none of the cases in which blood was present was there a lesion of the upper part of the gastro-intestinal tract or lungs that could account for it. Therefore, the natural deduction is that it unquestionably came from the lesions in the esophagus, which could well be responsible. For this reason, vomiting of blood indicates ulcerations of the esophagus in the known absence of gastro-intestinal or pulmonary lesions.

The vomiting of blood, although definite, usually did not seem to cause much concern, probably because of the serious condition of the patient at the time, when nothing could be done if the source had been ascertained, and also because the bleeding did not seem to be a serious complication, except in rare cases. Vomiting of blood caused faulty conclusions once. The patient had painless jaundice for five weeks following an attack of so-called ptomaine poisoning. Vomiting had

been present at intervals for four and a half weeks. In the hospital, the drainage from the duodenum did not reveal bile or blood, and a diagnosis of carcinoma of the head of the pancreas was made. The vomitus soon turned from dark to black (blood), and the possibility of the malignant lesion eroding into the stomach or duodenum with consequent bleeding was considered. At necropsy, it was found that this condition had not occurred. The bleeding unquestionably had proceeded from the diffuse ulcerations of the esophagus, which microscopically gave evidence of extensive inflammatory changes at the base of the acute, discrete, superficial, bile-stained ulcerations. This case suggests the clinical significance of the lesion.

In another case, esophageal bleeding following ulcerations into esophageal varices was the contributory cause of death. The patient was a woman, aged 68 years, who entered the clinic with a history of having had slight icterus for four months following an attack of influenza and colic of the gallbladder. Cholecystectomy and choledochostomy were performed for empyema of the gallbladder and stones in the gallbladder and ducts with impaction. The patient made an uneventful recovery until the fourth day, when she passed six tarry stools and became dyspneic. The pulse became rapid, and she failed rapidly. At necropsy, the stomach and intestines were found to be filled with blood. No source of the bleeding could be found other than acute ulcerative esophagitis, which had caused erosion of esophageal varices. Microscopic study proved the premise, for typical ulcerations were found, with erosions into the dilated vessels of the submucosa. This case also suggests the range of clinical importance of this pathologic lesion.

At necropsy, blood was found in the stomach in 7 cases. This varied in quantity from 200 cc. to so much that the stomach was described as full of blood. Here again, with the exception of a case in which an apparently clean gastro-enterostomy had been performed for a duodenal ulcer which did not bleed, no source for the blood except the esophagus could be found. The blood was described as having had the appearance of coffee grounds, or as having occurred in clots; therefore, there was no doubt as to its presence.

Trauma.—Trauma from intubation has been thought to be a factor in the production of these lesions, but close investigation of the case histories revealed that this could have occurred in only 30 cases, or about a third of the fatal cases. In the cases in which a tube had been passed into the esophagus, the lesions apparently were not different from those in which this had not been done. At first the linear ulcerations seemed to be the result of the intubation, but this again was not proved in this study. This warrants the conclusion that passage of a

tube is not an etiologic factor and may only incidentally be an aggravating one. One patient suffered considerable pain when the tube was passed.

Miscellaneous Symptoms.—Miscellaneous symptoms referable to the esophagus were present in 11 cases, and were grouped as follows: dysphagia, which occurred in 7 cases, and high epigastric pain or burning, which occurred in 4 cases. There were 5 cases in which there was severe to intractable hiccup, without peritonitis, pneumonia or other cause of phrenic irritation. Because of the fact that hiccup is occasionally an early, or even the first, symptom of carcinoma of the esophagus or of cardiospasm, it is probable that hiccup represents a symptom of ulceration of the esophagus. Therefore, I wish to include it and bring the number of cases with miscellaneous symptoms up to 16.

Dysphagia, although usually severe, can be qualified by the following instances: 1. One patient refused food because of the severe pain associated with swallowing. 2. "Burning all the way down the esophagus" was so severe in another patient's case that a laryngologist was called, and esophagoscopy was suggested. 3. A presumptive diagnosis of carcinoma of the esophagus was made once because of marked dysphagia, which was present at the time of the patient's admission to the hospital, and because of vomiting that had occurred previously. The patient died of uremia, which was caused by polycystic kidneys. 4. Lower substernal pain was so severe in 1 case that the question of disease of the gallbladder was considered. 5. Passage of a tube caused pain in 1 case in which dysphagia already was a symptom. 6. In 2 cases, burning, which was situated high in the epigastrium, was present without any apparent reason. Dysphagia or burning in the esophagus was present in only 11 of the 82 cases; this seems a very small proportion; on the other hand, when it is realized that these symptoms were complained of without the patients being questioned, it can be assumed that it was present in many more than 11 cases. There can be no doubt that ulceration, because of the symptoms mentioned, occurs without causing death and consequently does not represent merely a terminal affair; for this reason, the condition is of clinical significance.

It has been suggested that anesthesia has something to do with esophagitis. Moutier reported a case in which the patient had swallowed the anesthetic. However, esophagitis occurred in cases in which operation was not performed, and in the surgical cases the types of anesthesia were extremely variable; a large number of patients received local and spinal anesthesia. This suggests that anesthesia is not a predominant etiologic factor. In cases in which general anesthesia was used, the extent and type of ulceration were in no way different from those in other cases.

Perforation occurred in 2 cases, and in these there were no symptoms to indicate that the accident had occurred, and dysphagia was not present. Unquestionably, the perforation took place just before death, for there was no inflammatory reaction in the pleural cavity or mediastinal tissues. That the perforation was aided by the ulceration is proved by the fact that microscopic study of the edge of the perforation revealed inflammatory changes, which extended through the entire esophageal wall.

Mediastinitis, which was present in 1 case, produced dyspnea and pain in the upper part of the abdomen. The mediastinitis seemed to be secondary to the ulceration, for the entire wall of the esophagus was uniformly infiltrated beneath the ulceration, and the same process seemed to extend into the mediastinum. Because the patient had empyema, it might be inferred that this was the primary source of the mediastinitis, but there was no evidence of a communication between the pleura and the mediastinum. Also, the condition was really periesophageal, and involved only the lower third of the mediastinum, which was in the region in which the esophageal changes occurred.

COMMENT

As a result of the pathologic study, there seems to be no doubt that the term "acute ulcerative esophagitis" describes the condition which was found in these cases better than does the term "esophagomalacia," or "intravital softening." In all the cases there were, without a doubt, inflammatory changes. It seems imperative that the action of the gastric juice be accepted as contributing to the lesion, because gastric juice was present uniformly in the lower third of the esophagus. Vomiting and even nausea without vomiting, with relaxation of the cardiac spasm, seem extremely important as factors that permit gastric juice to come in contact with the esophagus.

It seems necessary that the patient should be debilitated, but not necessarily dying, before changes can occur in the esophagus. Debilitating and terminal states imply slowing of the circulation of the lower part of the esophagus, which normally is poor, and, therefore, loss of the normal resistance of esophageal tissue to trauma, and of its ability to regenerate. Thrombosis of small vessels has been suggested as a factor, but that it occurs has not been proved by microscopic study. Normally, as is known from experimental studies, the esophagus has good ability to repair any area that is traumatized, and the only conclusion which can be drawn is that in the cases which comprise this study the body was unable to carry on its work efficiently.

From a clinical standpoint, esophagitis should be recognized as a condition that is able to cause symptoms and to produce signs, as has

been brought out by close investigation of patients while they were under observation in the hospital. There is reason to believe that this condition is on the increase, for several years ago Verbrugghen did not find a significant number of cases. Of late, it is being found more often than it was found formerly, and there is no possibility that this frequency should have come about merely because it was not looked for as diligently in former years as it is at present. I have no explanation to offer for the increasing prevalence.

SUMMARY

The term "acute ulcerative esophagitis" is more appropriate and is suggested instead of the term "esophagomalacia," or "intravital softening," which was used previously.

The condition produces ulcerative changes in the lower part of the esophagus; these changes, both gross and microscopic, are described.

Acute ulcerative esophagitis is of clinical significance because of its symptoms and signs. It also seems to be more prevalent than it was formerly.

Peptic ulcer (so-called) of the esophagus was not encountered. Scars which would suggest its previous existence were not seen.

LYMPHOMATOSIS IN RELATION TO FOWL PARALYSIS

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CONTENTS

The etiology of lymphomatosis, the most common neoplasm of the domestic fowl, has been investigated by many workers familiar with

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both filtrable viruses and neoplasms, but without success. When Ellermann and Bang [1] observed that erythroleukosis and myeloblastic leukemia are caused by a filtrable virus they discovered a new filtrable virus as well as new types of leukosis in the domestic fowl. They believed that lymphoid leukosis was also caused by their virus, but Anderson and Bang [2] failed to transmit lymphoid leukosis, and the few instances of this disease occurring among the passages of the virus of Ellermann were probably spontaneous.[3]

Recent experiments indicate that lymphoid leukosis may be caused by several viruses.[4] The first virus (that of strain 2) which was shown to produce a lymphomatous neoplasm produced also endothelioma and myelocytomatosis. Lymphomatosis produced by this virus is associated with infiltration of nerves but with no clinical manifestations of paralysis.[4a] In subsequent experiments we have transmitted lymphomatosis associated with extensive infiltration of nerves and with clinical manifestations of fowl paralysis, but this disease has seldom been associated with leukemia, and never with endothelioma or myelocytomatosis (strains 5 and 6 [4b]). Successful transmission of this variety of lymphomatosis has already been reported by Pappenheimer, Dunn and Cone,[5] who have named it "neurolymphomatosis." Evidence will be presented here that these two types of lymphomatosis (strains 2 and 5) differ both etiologically and anatomically.

I. LYMPHOMATOSIS PRODUCED BY STRAIN 2

BLOOD CHANGES

Most lymphocytes that circulate in the blood of normal chickens are small and represent approximately 61 per cent of all leukocytes. Their cytoplasm is pale, and in some cells in preparations stained with a combination of Wright and Giemsa solutions, it contains minute azurophil granules. An occasional small lymphocyte with basophil cytoplasm may

1. (*a*) Ellermann, V., and Bang, O.: Centralbl. f. Bakt. **46**:1, 1908. (*b*) Ellermann, V.: The Leucosis of Fowls and Leucemia Problems, London, Gyldendal, 1921.

2. Anderson, C. W., and Bang, O.: Festskrift til Bernhard Bang, Copenhagen, 1928, p. 355.

3. (*a*) Furth, J.: Proc. Soc. Exper. Biol. & Med. **27**:155, 1929; J. Exper. Med. **53**:269, 1931. (*b*) Stubbs, E. L., and Furth, J.: ibid **53**:269, 1931. (*c*) Engelbreth-Holm, J.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:425, 1932. (*d*) Engelbreth-Holm, J., and Rothe Meyer, A.: Acta path. et microbiol. Scandinav. **9**:293, 1932.

4. (*a*) Furth, J.: J. Exper. Med. **58**:253, 1933; (*b*) Proc. Soc. Exper. Biol. & Med. **31**:921, 1934.

5. Pappenheimer, A. M.; Dunn, L. C., and Cone, V.: J. Exper. Med. **49**:63, 1929.

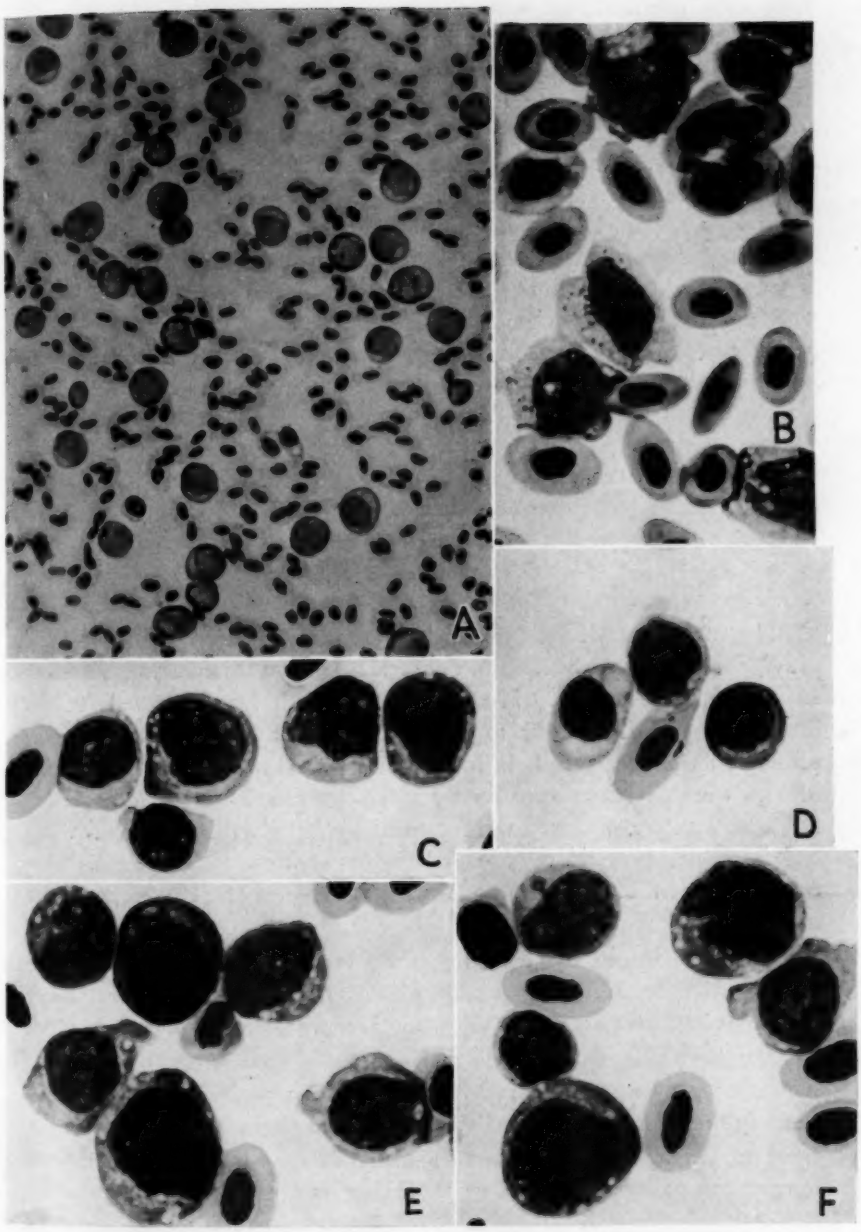

Fig. 1.—Blood cells in lymphatic leukemia produced by strain 2. The lymphocytes shown in *B* have minute azure granules. Transitional forms between large and small lymphocytes are seen in *C*. *D* shows a lymphocyte, an erythroblast and a thrombocyte. All blood smears were stained with Wright and Giemsa solutions. The magnifications are approximately: *A*, × 400; *B-F*, × 1000.

be seen in the blood of normal chickens, but basophil lymphocytes of medium and large size are absent. Lymphomatosis of strain 2 is characterized by the presence in the blood of numerous basophil lymphocytes of medium and large size (fig. 1 *A, B, C, E* and *F*). The cytoplasm of many of these cells contains vacuoles in some of which there are minute azurophil granules (fig. 1 *B*). There are continuous transitional forms between the large lymphocytes of strain 2 and normal small lymphocytes (fig 1 *C*). Erythroblasts can be readily distinguished from the lymphocytes of strain 2, as shown in figure 1 *D;* moreover, lymphatic leukemia produced by this strain is occasionally unassociated with anemia, notably after intramuscular inoculations with the large lymphocytes. Severe anemia almost invariably accompanies lymphatic leukemia produced by intravenous transmission, probably because of the early and extensive lymphomatous infiltration of the marrow. In most chickens with lymphomatosis of strain 2 the total number of leukocytes was slightly or moderately increased; in several chickens it exceeded 100,000 per cubic millimeter. The highest leukocyte count found was 1,200,000. Differential counts are shown in table 1.

ANATOMIC CHANGES

The tumors produced by intramuscular transmission of strain 2 are gray, somewhat yellowish, ill-defined neoplasm diffusely infiltrating adjacent muscle tissues. Most birds died with lymphomatosis of the internal organs from four to eight weeks after inoculation, when the tumors in the breast muscle measured from 1 to 4 cm. across. The tumors were of meaty consistency and were usually free from hemorrhage and necrosis. They were formed by large lymphocytes (fig. 2 *A* and *B*) which very readily invaded the blood stream. These cells were conspicuous in blood smears as early as two or three weeks after inoculation.

The liver, spleen and bone marrow were almost invariably the sites of extensive lymphomatous infiltrations; the thymus, kidneys, heart muscle, lungs, ovaries, nerves and ganglions were often infiltrated.

The liver was enlarged up to four times its normal size in most instances; gray mottling (fig. 3 *A*) caused by perivascular lymphomatous infiltration (fig. 4 *A*) distinguished lymphomatosis of this organ from erythroleukosis of strain 1, which is associated with a diffuse intravascular accumulation of erythroblasts or myeloblasts (leukostasis). Leukosis of strain 2 is characterized by extravascular lymphomatous infiltrations with (fig. 4 *A*) or without (fig. 4 *B*) leukostasis. In many chickens with lymphomatosis of strain 2 there were numerous gray lymphomas in the liver, measuring from 0.1 to 1 cm. in the largest

TABLE 1.—*Blood Counts and Differential Counts on Chickens with Lymphomatosis of Strain 2*

Chicken*	Date of Examination	Hemoglobin (Sahli), per Cent	Red Cell Count, Thousands	White Cell Count, Thousands	Immature Red Cells: Polychrome: Erythrocytes	Immature Red Cells: Polychrome: Erythroblasts	Immature Red Cells: Basophil Erythroblasts	Lymphocytes†: Mature, per Cent	Lymphocytes†: Basophil: Small, per Cent	Lymphocytes†: Basophil: Medium, per Cent	Lymphocytes†: Basophil: Large, per Cent	Granulocytes: Polymorphonuclears, per Cent	Granulocytes: Metamyelocytes, per Cent	Granulocytes: Mast Cells, per Cent	Monocytes, per Cent	Thrombocytes per 100 White Cells
2255	March 28	..	.....	.....	Few	Few	0	54.0	2.5	....	....	23.5	....	4.0	16.0	245
Presented spontaneous lymphomatosis	April 23	..	1,685	17.5	0	0	0	20.0	20.0	32.0	1.0	22.0	....	...	5.0	79
	April 27	..	1,198	130.0	0	0	0	3.0	15.0	74.0	1.0	4.0	....	...	3.0	58
2643	April 27	..	.....	.....	0	0	0	67.5	4.0	0.5	....	19.0	....	3.5	5.5	91
Inoculated April 28;	May 19	..	.....	.....	Few	Few	0	71.0	....	....	2.0	14.0	....	1.0	12.0	83
died June 9	June 18	44	1,705	160.0	Many	Many	0	8.0	2.0	30.0	31.0	24.0	....	2.0	3.0	28
2573	March 21	..	.....	.....	0	0	0	66.0	1.0	....	....	29.0	....	0.5	3.5	104
Inoculated April 23;	May 11	37	2,895	32.5	0	0	0	52.0	8.0	10.0	....	11.0	....	7.0	12.0	119
died May 18	May 18	..	.....	.....	0	0	0	19.0	11.0	28.0	12.0	15.0	1.0	2.0	12.0	109
2997	Aug. 9	..	.....	.....	0	0	0	75.0	0.5	....	....	16.0	0.5	0.0	8.0	81
Inoculated Aug. 11;	Sept. 17	35	1,840	63.0	Many	0	0	35.0	9.0	24.0	....	21.0	....	3.0	8.0	42
killed Oct. 14	Sept. 20	36	2,195	51.4	Few	0	0	32.0	5.0	21.0	1.0	21.0	2.0	8.0	10.0	32
	Oct. 14	38	1,325	35.0	Few	0	0	25.0	10.0	36.0	....	1.0	19.0	4.0	5.0	158
2976	Aug. 5	..	.....	.....	0	0	0	64.0	....	....	....	22.5	....	...	13.5	52
Inoculated Aug. 6;	Sept. 17	36	1,680	24.5	Very many	Many	0	42.0	35.0	4.0	....	13.0	1.0	2.0	3.0	9
died Oct. 3	Oct. 3	35	1,450	16.5	Many	Few	0	63.0	1.0	21.0	1.0	2.0	3.0	3.0	6.0	56
3202	Nov. 1	..	.....	.....	Few	0	0	64.0	....	1.0	....	17.5	3.0	4.5	10.0	41
Inoculated Oct. 27;	Nov. 11	..	.....	.....	Few	Few	0	80.0	1.0	2.0	1.0	10.0	....	2.0	4.0	80
died Nov. 23	Nov. 23	20	995	98.5	Many	Many	Few	2.0	85.0	11.0	....	....	....	1.0	1.0	3

* Fowl 2255 is the chicken in which strain 2 originated.[4a] Chicken 2643 affords an example of lymphatic leukemia produced by transmission. In chicken 2573 there was a moderate increase of lymphocytes unassociated with anemia, and in chicken 2997, a similar increase of lymphocytes associated with anemia. In fowl 2976 the number of white cells in the blood was not increased, but many of the leukocytes were basophil lymphocytes of medium size; fowl 3202 is an example of lymphatic leukemia with severe anemia produced by a cell-free virus.

† The differential counts are based on examination of dried smears containing 200 or more white cells. "Mature" designates small, not basophil, lymphocytes. Lymphocytes of an average diameter smaller than 9 microns are designated as "small," those measuring from 9 to 12 microns as "medium," and those larger than 12 microns as "large."

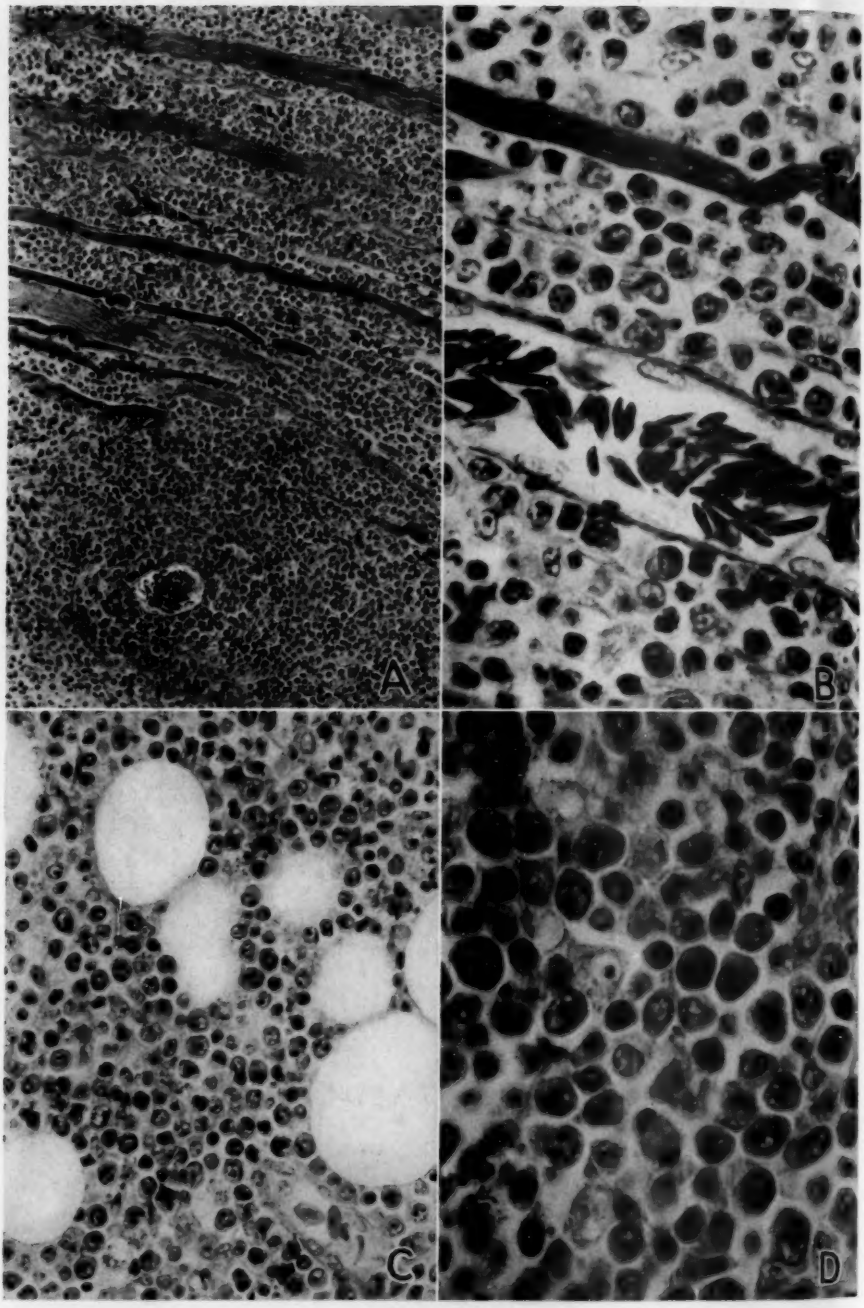

Fig. 2.—*A* and *B*, lymphomatosis of the inoculated breast muscle (strain 2); *C* and *D*, lymphomatous infiltration about the inoculated thymus. All the sections were stained with hematoxylin, eosin and azure II solutions. The magnifications are approximately: *A*, × 150; *B*, × 550; *C*, × 350; *D*, × 600.

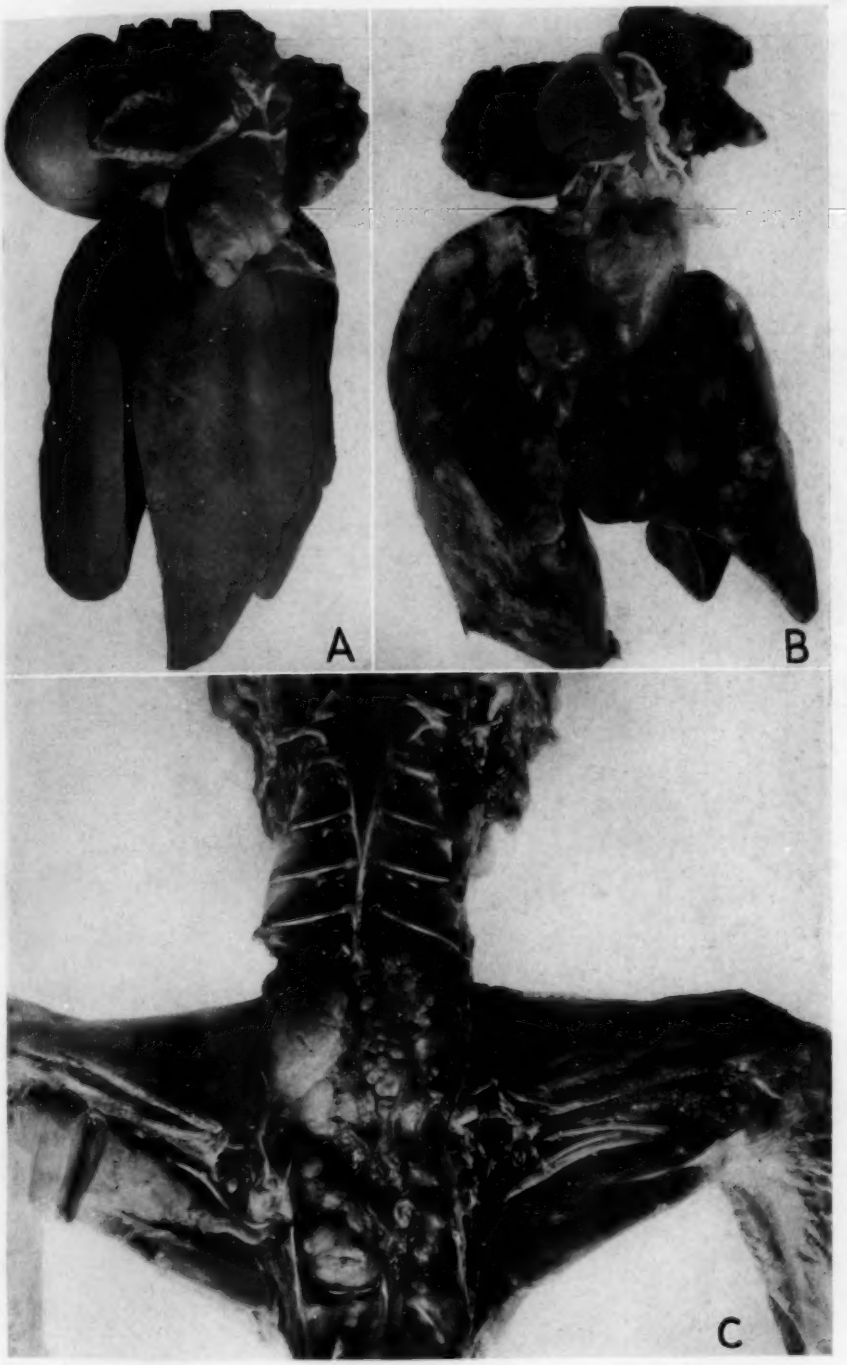

Fig. 3.—*A* and *B*, the liver, spleen, lung and heart of a chicken with lymphomatosis produced by strain 2. The liver shows, in *A*, minute gray spots, and in *B*, gray tumor nodules composed of large lymphocytes. Infiltration of the lung and enlargement of the spleen are shown in *A*. *C* shows tumor-like thickening of the inoculated right sciatic nerve associated with extensive infiltration of all lobes of the right kidney (strain 2).

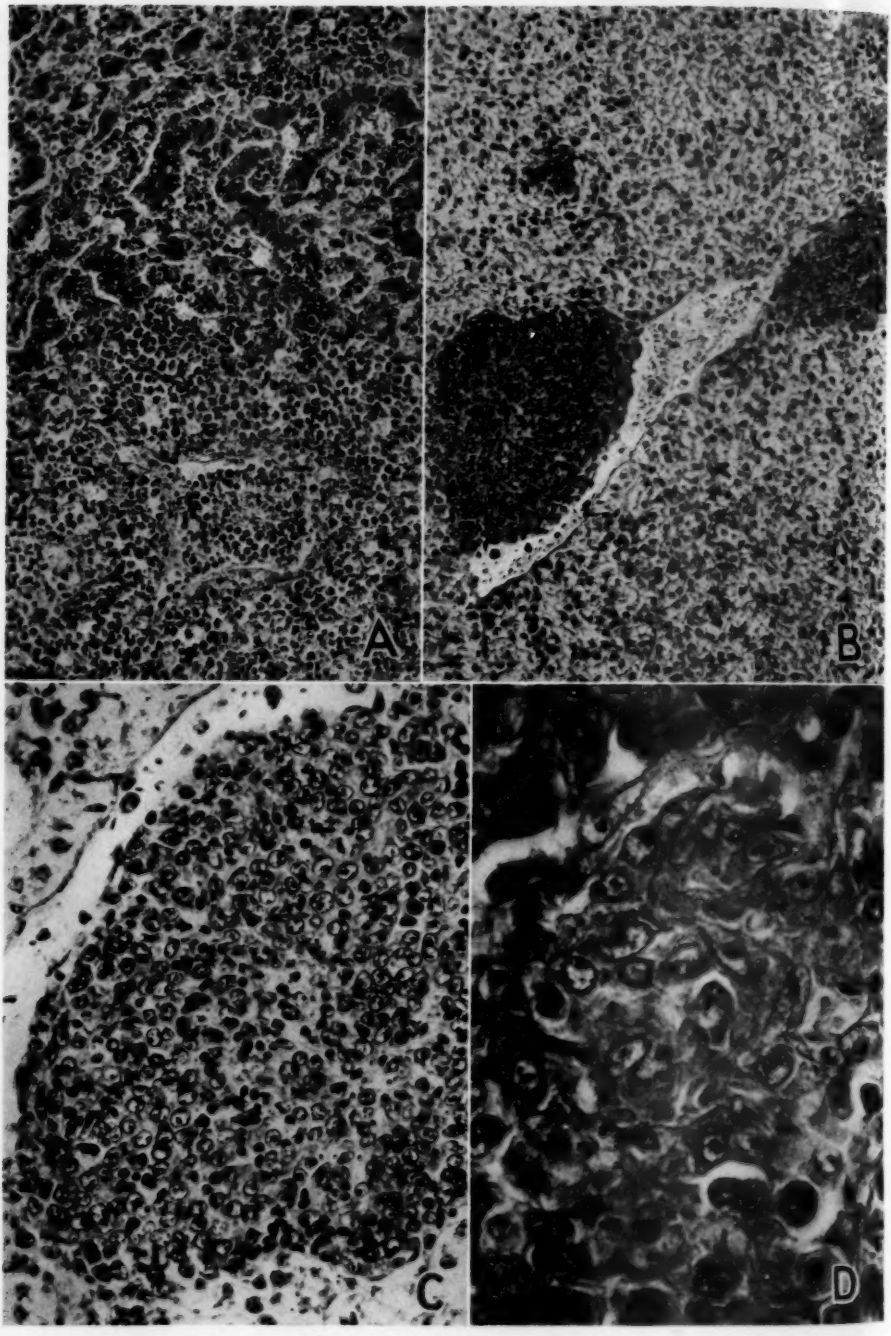

Fig. 4.—Lesions in the liver produced by strain 2. *A* shows perivascular and diffuse infiltrations. *B* and *C* show tumor nodules composed of cells many of which are probably endothelial or reticular. *D* shows a whorl-like arrangement of similar cells. The sections were stained with eosin and azure II solutions. The magnifications are approximately: *A* and *B*, × 180; *C*, × 400; *D*, × 700.

diameter (fig. 3 *A* and *B*). In a few chickens the extravascular infiltration was slight, and the liver appeared normal on gross examination. The presence of large numbers of basophil lymphocytes in the blood vessels distinguishes this disease from spontaneous lymphomatosis associated with great enlargement of the liver ("big liver" disease, hepatolymphomatosis [fig. 5 *A* and *B*]). In most instances of hepatolymphomatosis the bone marrow and spleen appear grossly normal. The few instances of this type of lymphomatosis occurring among the passages of strain 2 may have been spontaneous. Yet in at least one instance of lymphomatosis the autopsy observations were indistinguishable from those of spontaneous hepatolymphomatosis, although basophil lymphocytes were seen in the blood ante mortem.

The lymphomatous infiltrations in the kidney, heart and lung were similar to those of the liver, either affecting the organ diffusely or producing tumor nodules up to about 1 cm. in diameter. In the spleen the infiltration was localized in the follicles as well as in the pulp. Since the pulp also contained numerous erythrocytes it looked grayish red, while the follicles were gray; hence the gray mottling on gross examination. This organ varied in size from approximately normal to about ten times normal. The bone marrow was gray-red, "pyoid."

Considerable difficulty was often met with in distinguishing the large basophil lymphocytes of strain 2 from endothelial cells and basophil erythroblasts (figs. 4 *C* and 6 *A* and *C*). The large clear cells shown in these figures resemble closely those produced by the endothelioma virus of Begg and Murray,[6] but that virus does not produce lymphomatosis.

The frequent association of endothelial neoplasms with lymphomatosis is characteristic of strain 2, for it is not present in neurolymphomatosis[7] and in other varieties of lymphomatosis of chickens.[8] The histologic characteristics of endothelioma produced by this strain have been described.[9] Growth of cells, probably endothelial, occurring in association with lymphomatosis is shown in figures 4 *C* and *D* and 6 *A* and *C*. The virus of strain 2 also stimulates the growth of myelocytes,[10] and it has been suggested that the large basophil lymphocytes of strain 2 function as hemocytoblasts in the sense of Maximow.[9]

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6. Begg, A. M., and Murray, J. A.: Scient. Rep. Invest. Imp. Cancer Research Fund **9**:1, 1930.

7. Pappenheimer, A. M.; Dunn, L. C., and Seidlin, S. M.: J. Exper. Med. **49**:87, 1929.

8. (*a*) Tyzzer, E. E., and Ordway, T.: J. M. Research **21**:459, 1909. (*b*) Mathews, F. P., and Walkey, F. L.: J. Cancer Research **13**:383, 1929.

9. Furth, J.: J. Exper. Med. **59**:501, 1934.

10. Furth.[6a, 9]

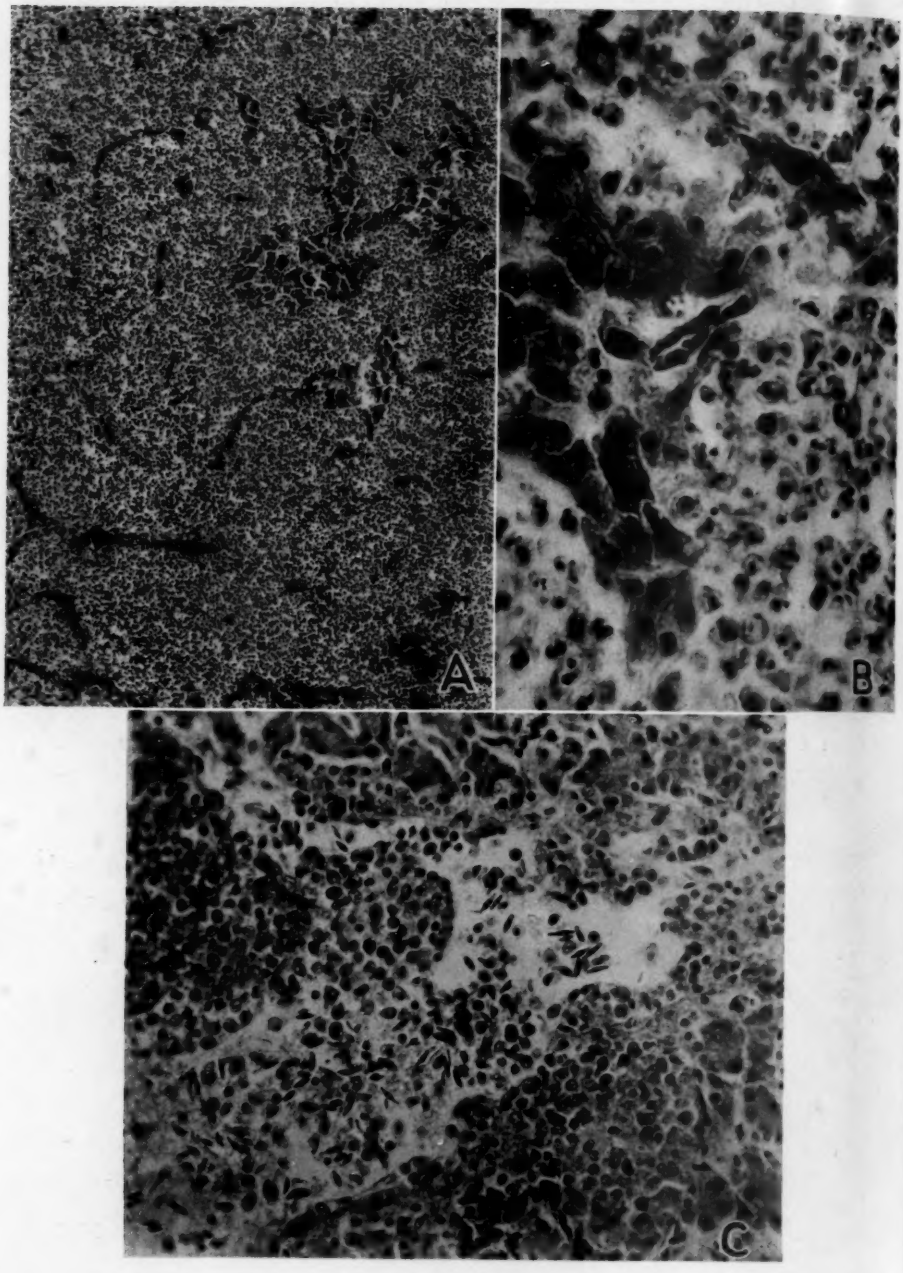

Fig. 5.—*A*, spontaneous hepatolymphomatosis ("big liver"); × 60. *B*, spontaneous hepatolymphomatosis with higher magnification; karyorrhexis of malignant lymphocytes; × 450. *C*, lymphomatous infiltration in the liver of a chicken with spontaneous neurolymphomatosis; × 300. The sections were stained with eosin and azure II solutions. The magnifications given are approximate.

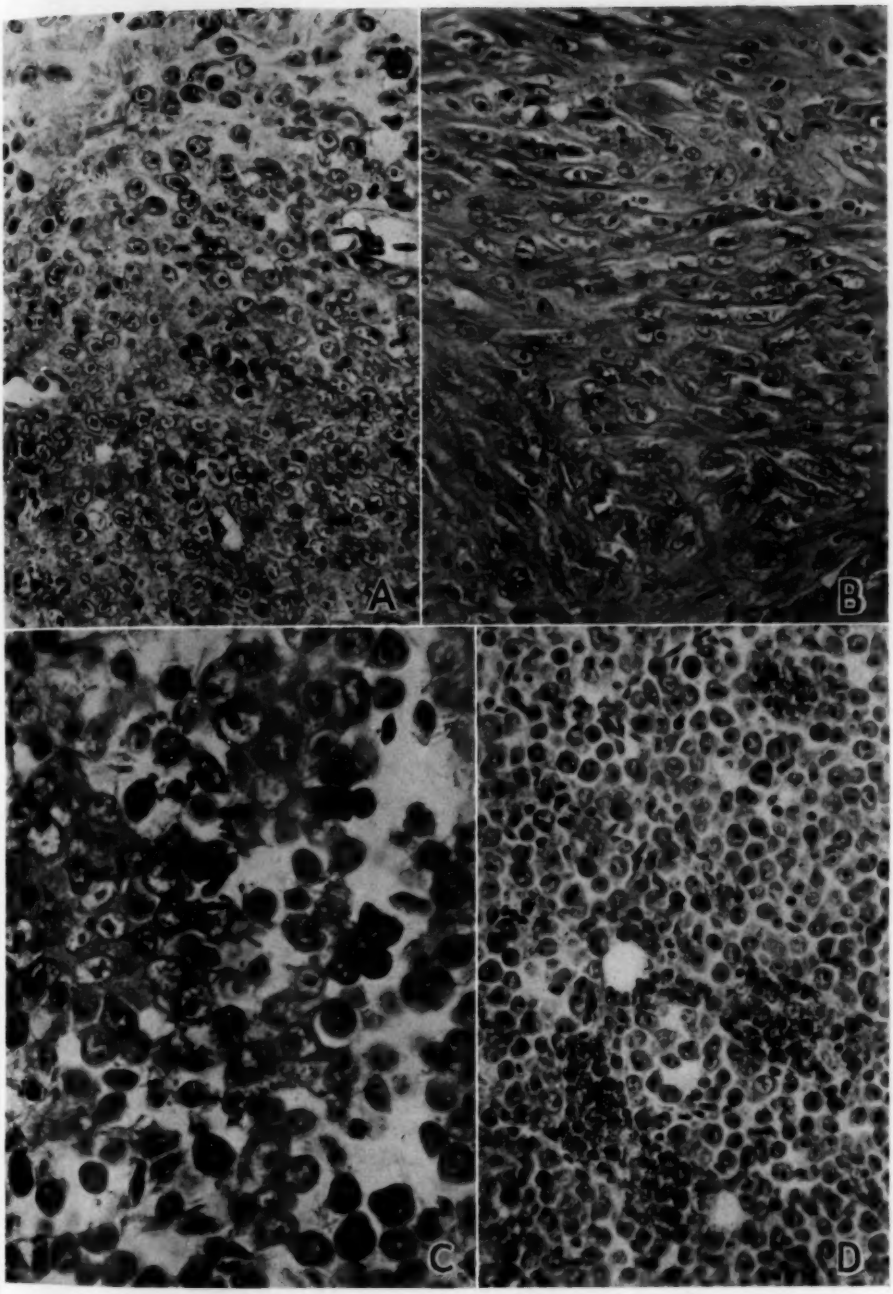

Fig. 6.—*A*, proliferation of cells, probably endothelial, in the breast muscle inoculated with strain 2; × 400. *B*, sarcoma-like growth in the inoculated breast muscle; × 450. *C*, growth in the kidney similar to that shown in *A*; × 700. *D*, hyperplasia of the bone marrow; × 350. *A*, *B*, *C* and *D* are from chickens inoculated with lymphomatosis strain 2. The sections were stained with eosin and azure II solutions. The magnifications given are approximate.

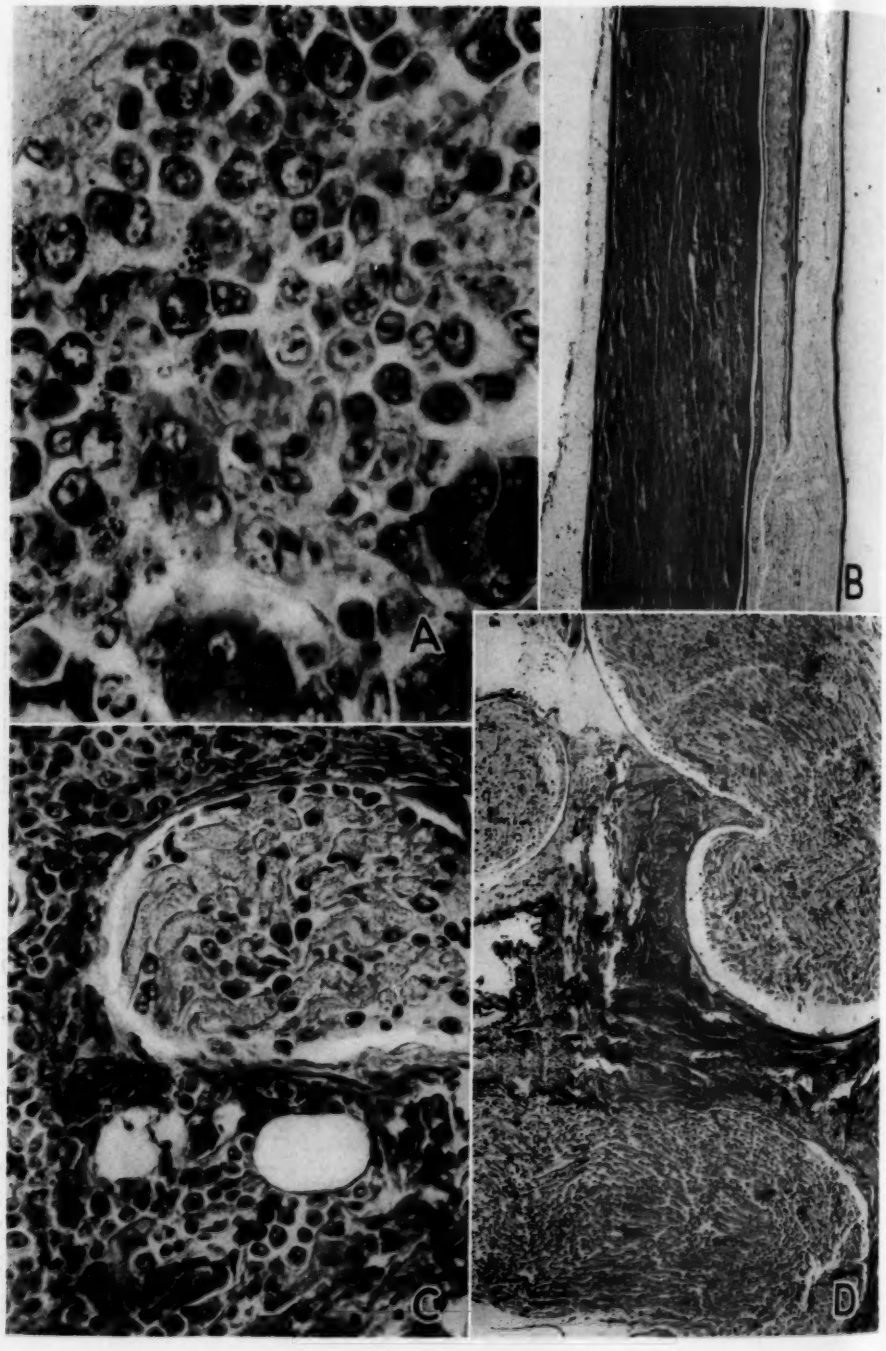

Fig. 7.—*A*, formation of myelocytes in the portal area of the liver; × 800. *B*, diffuse lymphomatous infiltration of one bundle of fibers of the sciatic nerve; adjacent nerve bundles are almost free from infiltration; × 20. *C*, infiltration of the vagus and surrounding tissue following intrathymic inoculation; × 400. *D*, infiltration of the brachial plexus following intravenous inoculation; × 80. *A*, *B*, *C* and *D* are from chickens with lymphomatosis of strain 2. The sections were stained with eosin and azure II solutions. The magnifications given are approximate.

Proliferation of myelocytes in association with hemocytoblasts is shown in figure *7 A*. The predominating type of large round cells that infiltrated the marrow (fig. *6 D*) could not be distinguished microscopically from basophil erythroblasts. However, erythroblasts seldom produce extravascular infiltration, and they do not infiltrate ganglions and nerves, even after intraneural introduction (see p. 38). Furthermore, in blood smears basophil lymphocytes can be readily distinguished from erythroblasts (fig. *1 D*). Lymphomatous infiltration of nerves, a frequent finding among chickens given injections of strain 2, is illustrated in fig. *7 B, C* and *D*. Post mortem large lymphocytes rapidly undergo pyknosis and karyorrhexis; repeated observations suggest that these changes in the lymphocytes precede similar changes in other cells of the organs which they infiltrate (e. g., liver cells, erythrocytes [fig. *5 A*]).

INTRAMUSCULAR TRANSMISSION WITH CELL-CONTAINING MATERIAL

The endothelioma of Begg and Murray,[6] all sarcomas of Rous [11] and the similar neoplasms studied in England under the Imperial Cancer Research Fund [12] readily produce tumors in the breast muscle. On the contrary, Ellermann and Bang,[1a] Jármai,[13] Engelbreth-Holm and Rothe Meyer [3c,d] and other workers, including ourselves, found no tumor formation at the site of inoculation when material from the common type of transmissible leukosis (like our strain 1) was injected into the muscle and subcutaneous tissue.

Oberling and Guérin [14] suggested that our failure to discover the ability of the virus of Ellermann to produce tumors was due to the fact that we used the intravenous route for inoculation. We have made numerous unsuccessful attempts to produce tumors with leukotic tissues of strain 1 by inoculations other than intravenous.[15] In some of these experiments leukotic cells of strain 1 were embedded in plasma clot preceding the inoculation in order to facilitate tumor formation at the site of inoculation. Since the percentage of successful inoculations was smaller after subcutaneous and intramuscular injections than after intravenous injections, we decided to transfer strain 1 by the intravenous route only. The observations of Oberling and Guérin induced us to test again whether leukosis of strain 1 produced tumors in the inoculated thymus and breast muscle, and we found that it

11. Rous, P.: J. A. M. A. **56**:198, 1911. Rous, P.; Murphy, James, and Tytler, W. H.: ibid. **59**:1793, 1912. Rous, P., and Lange, L. B.: J. Exper. Med. **18**:651, 1913.

12. Foulds, L.: Scient. Rep. Invest. Imp. Cancer Research Fund **11**:1, 1934.

13. Jármai, K.: Arch. f. Wissensch. u. prakt. Tierh. **62**:113, 1930; **65**:46, 1932.

14. Oberling, C., and Guérin, M.: Bull. Assoc. franç. étude du cancer **20**:180 and 326, 1933.

15. Furth.[3a] Stubbs, E. L., and Furth, J.: Unpublished work.

did not. The characteristics of this strain are the same now as they were four years ago,[3a] and it still produces erythroleukosis and myeloblastic leukemia but does not produce endothelioma or Rous sarcoma.

Leukosis strain 2, in contrast with the common varieties of leukosis strains, produces extensive extravascular infiltration or tumors composed of myelocytes or cells like large lymphocytes; it also produces endothelioma. It may therefore be supposed that inoculation with cells of strain 2 would produce lymphomatosis, myelomatosis or endothelial growth in the breast muscle, depending mainly on the type of cell introduced.

Series 1.—The first series of intramuscular inoculations, reviewed in a former report,[4a] were unsuccessful. In these experiments, made between April and July 1932, twenty-nine chickens were inoculated intramuscularly, and no tumors developed at the sites of injection. One chicken died of general lymphomatosis, ten died of intercurrent disease within one or two months after inoculation, and eighteen remained healthy.

Series 2.—Seven months later further attempts were made to produce tumors in the thymus, where spontaneous leukotic tumors of all kinds are often seen, and in the breast muscle, in which Rous sarcoma and endothelioma grow readily.

In ten experiments, thirty chickens were given injections of blood cells or tumor tissue composed of large lymphocytes, myelocytes or neoplastic endothelium. Most of the injections were made into the breast muscle, subcutaneous tissue or thymus, and a few into the region of the kidney. Leukosis developed in twenty-four (80 per cent) of the inoculated birds, and in twelve of them grossly visible infiltrations or tumors were found in the muscle or thymus at the site of inoculation. In two chickens there was, in addition, a sarcoma-like growth (fig. 6 *B*) which, although it had the character of a malignant growth, remained small, barely detectable with the naked eye, and limited to the site of injection. The last-mentioned characteristics distinguish it from Rous sarcoma, which, even if mixed with the agent of leukosis, grows profusely in the breast muscle receiving the injection and often metastasizes to distant organs. In seven of these experiments the material used for intramuscular injection was also injected intravenously, and leukosis developed in twelve (63 per cent) of nineteen chickens thus inoculated.

The first of these experiments, made with cells of a tumor composed mainly of myelocytes, had the following result: Three of the four birds given intramuscular injections acquired systemic leukosis, chiefly lymphomatosis, and in one of these there was extensive infiltration of the inoculated breast muscle by cells like large lymphocytes (fig. 2 *A* and *B*). In another experiment two of the three

chickens given injections of a cell suspension of myelocytoma associated with endothelioma presented infiltration of the inoculated thymus (fig. 2 *C* and *D*) and breast muscle by large lymphocytes. The vast number of mitoses (as many as 15 in a field magnified 400 times) gave evidence of the unrestricted growth of the large lymphocytes of strain 2. Since the blood-forming organs of one of these two birds showed only slight alterations, and mitosis among the lymphocytes was abundant at the site of injection, it is probable that these cells grew unrestrictedly in the inoculated thymus and breast muscle. It is noteworthy that the tumor tissue injected, composed mainly of myelocytes and endothelial tumor cells, contained few large lymphocytes.

The largest tumor that developed in a thymus lobe receiving an injection measured 3 by 2.5 by 2 cm. fifty-two days after inoculation, when the bird was killed for study. Thymus lobes not receiving injections and the liver, spleen and bone marrow were normal. These observations further support the view that the tumor at the site of inoculation originated in the implanted neoplastic cells.

In another experiment minced tissue of an ovarian tumor (endothelioma with giant cells) mixed with minced tissue of a hepatic tumor (lymphoma) of the same bird was injected into both the right breast and a lobe of the thymus in six chickens. Two of these were killed four days and another two were killed ten days after inoculation for the purpose of studying the early lesions. Four and ten days after inoculation the alterations were localized to the sites of injection and consisted of apparently neoplastic growth of large lymphocytes; these cells evidently originated from those introduced. In two chickens examined thirty-one and thirty-eight days after inoculation small sarcoma-like growths (fig. 6 *B*) were found at the sites of injection. These growths remained localized, and the distant lesions were those of leukosis, mainly lymphomatosis. The significance of these sarcoma-like lesions remains obscure. They were never seen again among the large number of birds successfully inoculated in a similar manner.

These experiments have shown that lymphomatosis may be produced in the breast muscle with leukosis strain 2.

Series 3.—After these partially successful attempts we succeeded in producing readily transmissible lymphomatous tumors in the breast muscle (fig. 8). Lymphomatous tumors composed of large lymphocytes developed in almost every inoculated bird. This was followed by invasion of the blood (lymphatic leukemia) and metastatic infiltration in numerous organs, mainly the bone marrow, liver, spleen, ovary, peripheral nerves and ganglions.

Chicken 3870 (strain 2) had numerous lymphomatous tumors in the liver and kidney. A suspension of particles of these tumors was injected into the thymus and breast muscle in three chickens, two of which acquired lymphomatous tumors at the sites of injection and died with blood changes of leukemia associated with lymphomas of numerous organs. The results of the subpassages are shown in figure 8. The microscopic changes in chicken 4339 were suggestive of neurolymphomatosis (see p. 23).

Summary.—Strain 2 transmitted intramuscularly with material containing lymphocytes produces lymphomatous infiltration or tumors in the muscles receiving injections. The following observations indicate

that these neoplasms are the result of unrestricted multiplication of the large lymphocytes that were introduced: Mitosis is abundant among the large lymphocytes; proliferative changes in normal cells are absent at the site of injection; the lymphocytes at the site of infiltration have the same cytologic characteristics as those introduced; the blood-forming organs of chickens with lymphomatous tumors in the inoculated breast muscle show no alterations at the early stage of the disease.

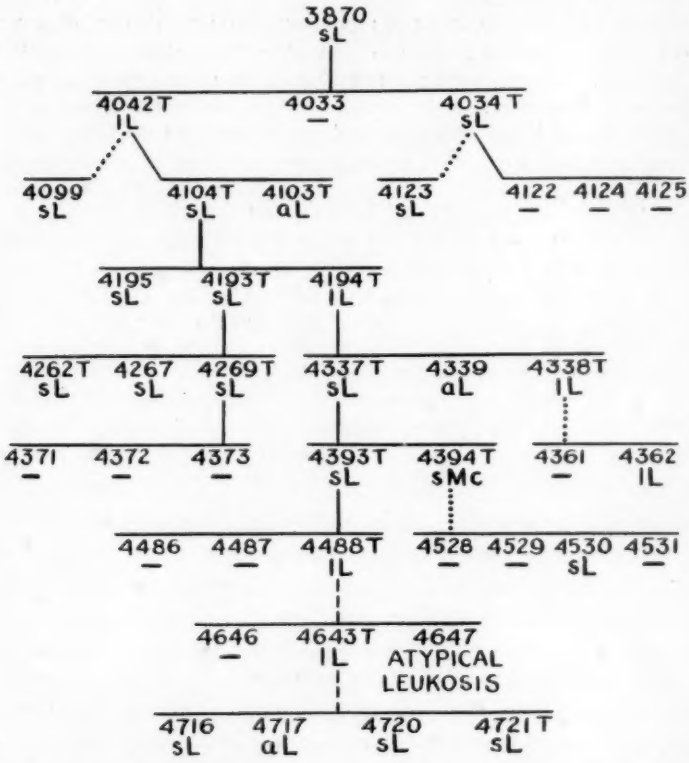

Fig. 8.—Transmission experiments with lymphomatosis strain 2. Each number is that of a chicken. A vertical solid line indicates intramuscular injection; a broken vertical line, intramuscular and intravenous injections, and a dotted line, intravenous injection. *sL* signifies subleukemic lymphomatosis; *T*, tumor in the inoculated breast muscle; *lL*, leukemic lymphomatosis; *aL*, aleukemic lymphomatosis; *sMc*, subleukemic myelocytomatosis; —, inoculation unsuccessful.

ATTEMPTS AT INTRAMUSCULAR TRANSMISSION WITH CELL-FREE VIRUS

The experiments described in the preceding section show that the large lymphocytes of strain 2 behave like malignant cells, undergo unrestricted multiplication at the site of introduction, invade the blood

stream, and produce metastases. They differ from neoplastic cells in mammals in that they elaborate a filtrable agent that transforms normal lymphocytes into malignant ones. The following experiments show that the cell-free virus of strain 2, in contrast to the virus of Rous sarcomas and that of the endothelioma of Begg and Murray, does not produce neoplasms in muscles receiving injections. The chickens given intramuscular injections of cell-free material either remained healthy or acquired the systemic disease with no infiltration at the site of injection.

Cell-free material of lymphomatous chickens was tested and in no instance produced a tumor at the site of injection. In most experiments dried blood or tumor tissue was used for the injection; in two experiments dried plasma and in one frozen and thawed blood were used. In seven of the eight instances of leukosis produced by the cell-free virus large lymphocytes were numerous in the blood. One chicken had leukemic myelocytomatosis.

TABLE 2.—*Data on Injections of Cell-Free Virus of Strain 2*

Material Injected	Route of Injection*	Chickens Inoculated	Chickens with Leukosis
Cell-free	Intrav. and Intram.	29	4
Cell-free	Intrav.	4	1
Cell-free, repeated injections with material from several chickens	Intrav. and intram.	6	3
Cell-containing	Intrav.	13	9
Cell-containing	Intrav. and intram.	4	4

* "Intrav." and "intram." are abbreviations for intravenous and intramuscular.

Summary.—The virus of strain 2 must reach blood-forming tissues in order to produce leukosis. It does not produce tumors in the muscle tissue receiving the injection.

INTRANEURAL TRANSMISSION

Strain 2 has an affinity for nerve tissues [4a] and, as the following experiments show, is readily transmitted by intraneural inoculations:

Experiment 1.—Intraneural injection of lymphomatous tumor tissue gave neurolymphomatosis in four chickens that died or were killed and examined at twenty-five, thirty, thirty-two and thirty-seven days, respectively, after inoculation (average, killed or died at thirty days).

Experiment 2.—Intraneural injection of tumor tissue and intravenous injection of 0.1 cc. of blood gave neurolymphomatosis in one chicken that died and was examined at twenty-five days.

Experiment 3.—Intramuscular injection of lymphomatous tumor tissue and intravenous injection of 0.1 cc. of blood gave neurolymphomatosis in four chickens that were killed or died and were examined at twenty-seven, thirty-two, forty-two and forty-nine days, respectively, after inoculation (average, killed or died at thirty-seven and a half days).

In these three experiments all inoculations were successful.

The experiments show that strain 2 can be readily transmitted by intraneural injection. The inoculated nerves became greatly thickened, and there was extensive lymphomatous infiltration about the nerve extending by continuity into the adjacent muscle tissue (fig. 3 *C*). Subsequently, large basophil lymphocytes and erythroblasts appeared in the blood.

Another experiment with fewer animals yielded similar results.

The neurolymphomatosis strains 5 and 6 that will be described in part II do not produce anemia and are seldom associated with lymphatic leukemia.

Summary.—The intraneural route is very favorable for the transmission of lymphomatosis strain 2, and the large lymphocytes of strain 2 proliferate in and about the inoculated nerves.

II. NEUROLYMPHOMATOSIS (FOWL PARALYSIS) PRODUCED BY STRAINS 5 AND 6

In 1907 Marek,[16] in Hungary, described a disease characterized by extensive round cell infiltration of the peripheral nerves and of the posterior root ganglions and named it "neuritis interstitialis." Two types of nontraumatic paralysis have been since recognized in the bird: (*a*) nutritional paralysis due to noninflammatory, degenerative changes caused by vitamin B deficiency (Eijkmann) and (*b*) Marek's *Geflügellähme*.

"Infectious polyneuritis," "neuromyelitis," "range paralysis," "fowl paralysis," and "neurolymphomatosis" are synonymous terms proposed for Marek's paralysis, of which only the term "neurolymphomatosis" will be used in this report. Neurolymphomatosis has been observed in the United States and Canada, in several countries of Europe, in Africa and in Japan.[17] It is now recognized as one of the most common fatal diseases of young chickens from 3 to 11 months of age.

Failure of experimental transmission of the disease has been reported by numerous workers, although most of them have expressed the belief that the causative agent is a virus.

Successful transmission of neurolymphomatosis was first described by Van der Walle and Winkler-Junius, but the evidence presented by these, as well as by several other workers, is not sufficient to support their conclusions.[5] The first experiments that strongly suggested the transmissibility of neurolymphomatosis were made by Pappenheimer, Dunn and Seidlin[7]: Paralysis occurred in 26 per cent of their experimental birds as compared with 8 per cent of their uninoculated controls.

16. Marek, J.: Deutsche tierärztl. Wchnschr. **15**:417, 1907.

17. The literature on neurolymphomatosis has recently been reviewed by Jungherr, E. Storrs Agric. Exper. Stat. Bull., no. 200, 1934.

Dalling and Warrack [18] observed that 27 per cent of the inoculated chickens acquired paralysis, as compared with 2.5 per cent of the uninoculated controls. It is noteworthy, however, that the death rate, due mainly to coccidiosis, was 38.5 per cent among their inoculated chickens and only 3.8 per cent among their uninoculated controls, but this is obviously no evidence that coccidiosis was influenced by the inoculations. Transmission experiments of Seifried [19] made on a small number of chickens suggested that neurolymphomatosis is transmissible by feeding, but this has not been confirmed.

The etiology of neurolymphomatosis and its relation to leukosis of chickens are subjects of much controversy.

Ellermann and Bang and most workers who subsequently studied leukosis of chickens did not observe paralysis among their experimental birds; most of those who studied neurolymphomatosis, on the contrary, noted the frequent association of fowl paralysis with lymphomatous tumors of the viscera.[7] Our experiments have shown that there are several agents that produce leukosis; one (that of strain 1) causes erythroleukosis and myeloid leukosis and is unassociated with infiltration in the nervous system, while another (that of strain 2) produces lymphomatosis with infiltration in the nervous system. Strain 2 almost invariably causes severe anemia, extensive lymphomatous infiltration of the bone marrow and the appearance of numerous large basophil lymphocytes in the blood, but none of these alterations are known to occur in association with neurolymphomatosis. These differences have suggested that the causative agent of fowl paralysis is not identical with any of the known strains of leukosis, and this suggestion is confirmed by the experiments described here.

Although several workers found a greater incidence of paralysis among their experimental birds than among uninoculated controls, none described a strain that could be passed readily from diseased to healthy chickens and studied in successive passages. Since the filtrability of some strains of avian tumors and of avian leukosis is demonstrated with difficulty, experiments to demonstrate the filtrable cause of the disease were not undertaken until the disease had been transmitted by viable cells. We have succeeded in isolating strains of neurolymphomatosis with the technic used for grafting malignant cells of mammals. The disease could not be transmitted with cell-free material in spite of the ease with which it was transmitted by material containing viable lymphocytes. In transmission experiments neurolymphomatosis behaved like a neoplasm in which the malignant cells are lymphocytes with special affinity for the peripheral nerves.

18. Dalling, T., and Warrack, G. H.: Atti de V. Congresso Mondiale di Pollicoltura, 1933, no. 90.

19. Seifried, O.: Arch. f. wissensch. u. prakt. Tierh. **62**:209, 1930.

MATERIAL OF STUDY AND PROCEDURES

All chickens observed with spontaneous neurolymphomatosis and most chickens used in the experimental work were Barred Rocks; also a few White Leghorns tested were found to be susceptible to the disease.[20] The experimental birds were from 4 to 15 weeks of age when first given injections.

Infiltrated nerves and lymphomatous tumors were cut up in Locke's or Tyrode's solution and filtered through a small piece of cotton for intraneural and intravenous inoculation. Intraneural injections were made in the exposed sciatic or ulnar nerves of anesthetized birds. The whole blood used for injection contained approximately one tenth of its volume of heparin solution (0.1 per cent). The amount injected was estimated to be approximately from 0.01 to 0.02 cc.

At the beginning of the experiments only those birds that showed gross evidence of neurolymphomatosis were studied microscopically, so that several of the chickens given in this report as not showing neurolymphomatosis may have had microscopic lesions of the disease. Microscopic examination of nerves often disclosed unquestionable neurolymphomatosis that was not detected on gross examination. Since the location of infiltration varied greatly and usually only from two to five nerves and the sympathetic ganglion near the adrenal were taken for microscopic examination, several cases of neurolymphomatosis may have escaped detection.

The incidence of death due to conditions other than fowl paralysis was high among the experimental birds. Most of the birds that appeared healthy and were killed five months after inoculation when the experiment was brought to an end were not examined microscopically. Some chickens were killed in the terminal stage of paralysis, and a smaller number were killed to combat small spontaneous epidemics, such as fowl pox, coryza or coccidiosis.

BLOOD CHANGES

Most workers have failed to observe blood changes in association with neurolymphomatosis, but Johnson [21] reported an increase of monocytes and mast cells and the presence of "budding lymphocytes" in the blood.

With rare exceptions the blood of our chickens with neurolymphomatosis appeared normal during the entire course of illness. There was a moderate or great increase of lymphocytes in the blood of chicken 3878 in which strain 5 originated and in chickens 3831 and 4570 inoculated with this strain (table 3). Almost all of these lymphocytes were small, but many of them were basophil (fig. 9 *B* and *C*). Lymphatic leukemia occurred in a fourth chicken (3948) on invasion of the blood by basophil lymphocytes of medium and large size (fig. 9 *D* and *E*). It is possible that this was a spontaneous disease. Erythroblasts were never found in the blood of chickens with neurolymphomatosis. All three chickens with small cell lymphatic leukemia had extensive neural and visceral lymphomatosis.

Differential counts were made on the blood of ten chickens with transmitted lymphomatosis the blood of which appeared normal on routine

20. The spontaneous disease is known to affect all breeds of chickens.

21. Johnson, E. P.: Virginia Agric. Exper. Stat. Bull., no. 44, 1932; 1934, no. 56; J. Am. Vet. M. A. **83**:325, 1933.

TABLE 3.—*Differential Counts on the Blood of Chickens with Neurolymphomatosis*

Chicken	Date of Examination	White Cell Count	Lymphocytes*				Granulocytes			Monocytes, per Cent	Thrombocytes per 100 White Cells
			Mature, per Cent	Basophil			Polymorphonuclears, per Cent	Metamyelocytes, per Cent	Mast Cells, per Cent		
				Small, per Cent	Medium, per Cent	Large, per Cent					
3878. Presented spontaneous neurolymphomatosis	July 11	145,000	80.0	4.0	2.0	....	5.0	....	...	....	0.5
3831. Inoculated July 18;	June 30	Normal	58.0	....	....	....	35.0	....	1.5	5.5	25.0
died Sept. 15	Aug. 22	Moderate increase	11.0	11.5	....	....	35.0	21.0	5.0	16.5	22.0
	Sept. 15	Moderate increase	35.0	45.0	....	....	2.5	12.0	0.5	5.0	41.0
4570. Inoculated May 28;	March 27	Normal	54.0	3.0	....	....	34.0	2.0	3.0	4.0	120.0
died June 28	June 28	Moderate increase	35.0	54.0	2.0	....	7.0	....	1.5	0.5	14.0
3948. Inoculated Sept. 21;	Sept. 22	Moderate increase	29.0	1.0	....	....	58.0	....	3.0	9.0	24.0
died Nov. 17	Oct. 23	Moderate increase	18.0	8.5	....	....	42.0	6.0	...	25.5	36.0
	Nov. 17	Great increase	1.0	15.0	46.0	13.0	9.0	2.5	0.5	13.0	30.0
Average of ten chickens:											
Before inoculation.....	.....	.....	61.5	5.0	....	....	22.7	0.3	3.2	7.3	36.0
At height of illness.....	.....	.....	31.3	14.3	0.3	0.1	38.0	2.0	2.7	11.3	58.0

* For explanation of terms, see table 1.

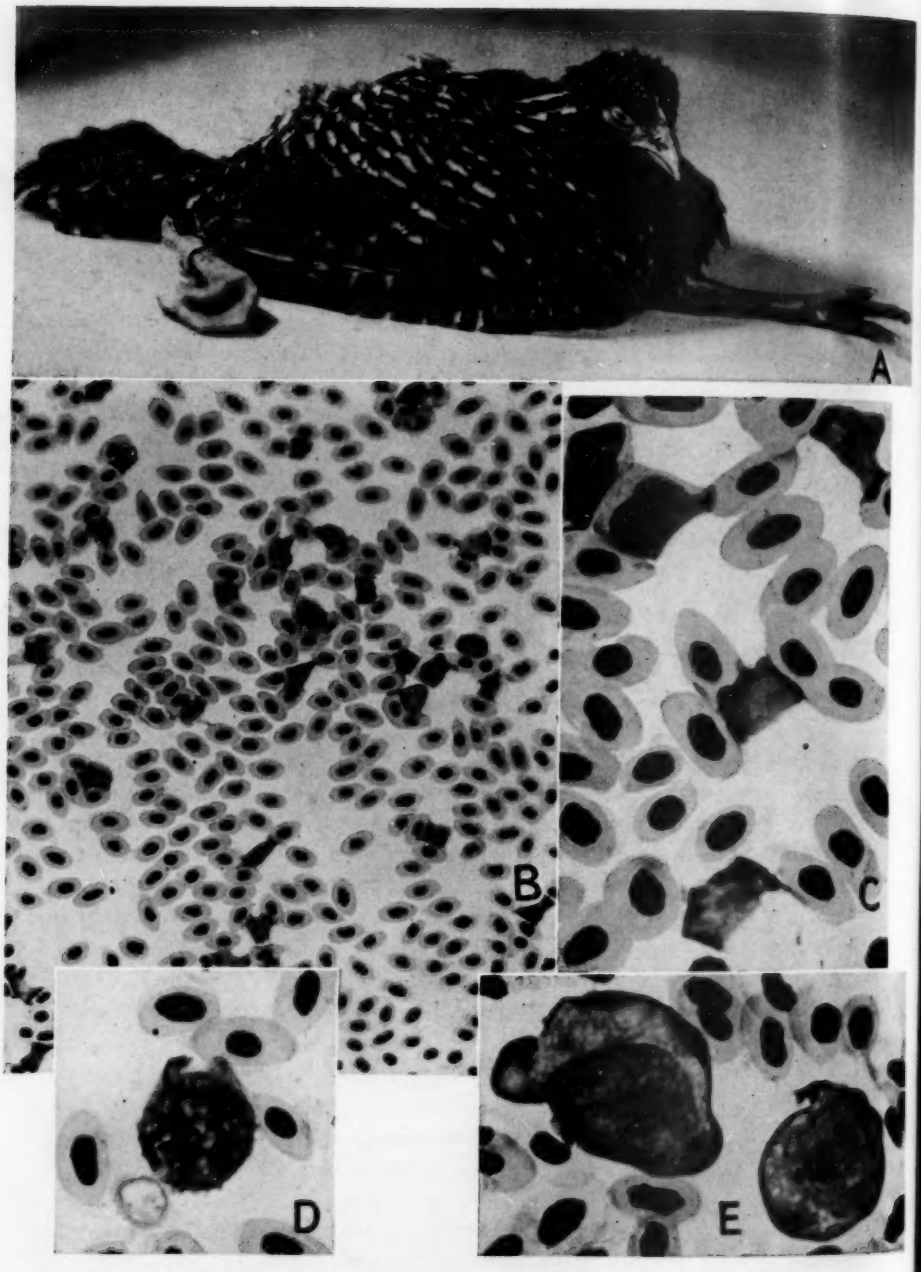

Fig. 9.—*A*, characteristic posture of a fowl with paralysis of the legs. *B*, lymphoid leukemia in a fowl (3878) with spontaneous neurolymphomatosis; × 400. *C*, higher magnification of the lymphocytes shown in *B*; × 1100. *D*, a mitotic figure. *E*, two lymphocytes from the blood smear of a fowl with large cell lymphoid leukemia (3948, strain 5); × 1100. The blood smears were stained with Wright and Giemsa solutions. The magnifications given are approximate.

examination. Eight showed a conspicuous increase in the percentage of small basophil lymphocytes; the average figure for these cells rose from 5 per cent before inoculation to 14.3 per cent shortly before death (table 3). The small basophil lymphocytes in the blood of paralyzed chickens were indistinguishable from the similar cells of normal chicken blood.

ANATOMIC CHANGES

The anatomic changes associated with neurolymphomatosis have been well described by numerous workers. We refer particularly to the articles of Marek [16] and Pappenheimer, Dunn and Cone [5] and mention only observations that deviate from or add to those of previous workers. The changes produced by strains 5 and 6 are indistinguishable from those associated with the spontaneous disease. The characteristic posture of a chicken with neurolymphomatosis of the sciatic nerves and lumbosacral plexuses is shown in figure 9 *A*.

The basic alteration in neurolymphomatosis consists of infiltration of the peripheral nerves (fig. 10 *B, C* and *D*) and ganglions (fig. 11 *A* and *B*) by cells like lymphocytes. The character of the infiltration is in most instances that of a lymphomatous neoplasm. The degenerative and inflammatory changes with which lymphomatosis is associated may be regarded as secondary. Several nerves are usually involved but to a variable extent; almost any peripheral nerve may be involved; the vagus (fig. 12 *A*), the brachial plexus (fig. 12 *D*), the intercostal, splanchnic and lumbosacral plexuses (fig. 12 *B*) and their branches are common sites of neurolymphomatosis. The diagnosis can be made in the majority of instances from the thickening, grayish discoloration and loss of the normal cross-striations of nerves. In a small number of instances the changes are scant, and the disease may be overlooked on gross examination. Since microscopic examination of the entire peripheral nervous system is not feasible, it is impossible to exclude the presence of mild lesions of neurolymphomatosis in any bird. The lymphocytes are of small or medium size, like normal lymphocytes in appearance.

Usually the small, occasionally the medium-sized, lymphocytes are predominant. Among the lymphocytes of medium size mitosis is abundant. The absence of polymorphonuclear leukocytes is significant. The lymphocytes are found scattered diffusely between the nerve fibers, but often there is a conspicuous perivascular cuffing in and about the nerve. The diagnosis of fully developed neurolymphomatosis offers no difficulties; the meaning of mild lymphocytic infiltration is doubtful. Lymphoid infiltration, not neoplastic in character, is often associated with the formation of true germinal centers (fig. 13 *C* and *D*) showing histologic evidence of lymphocyte formation (fig. 13 *D*). Formation of germinal centers, absent in lymphomatosis, may be regarded as evidence of orderly lymphocyte formation.

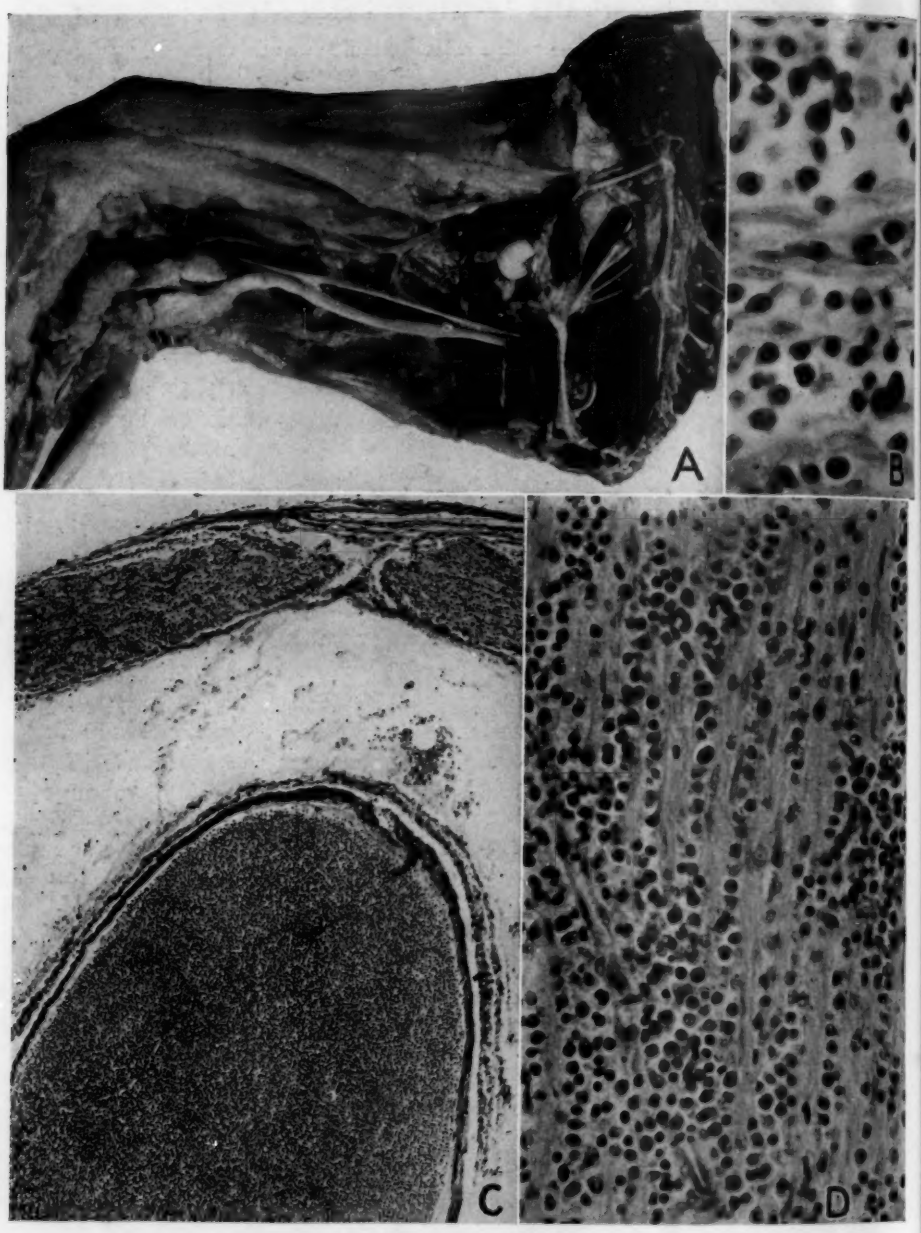

Fig. 10.—*A*, thickening of the inoculated sciatic nerve (strain 5). *B*, *C* and *D*, infiltration of nerves in spontaneous neurolymphomatosis. The sections were stained with eosin and azure II solutions. The magnifications are approximately: *B*, × 450; *C*, × 60; *D*, × 250.

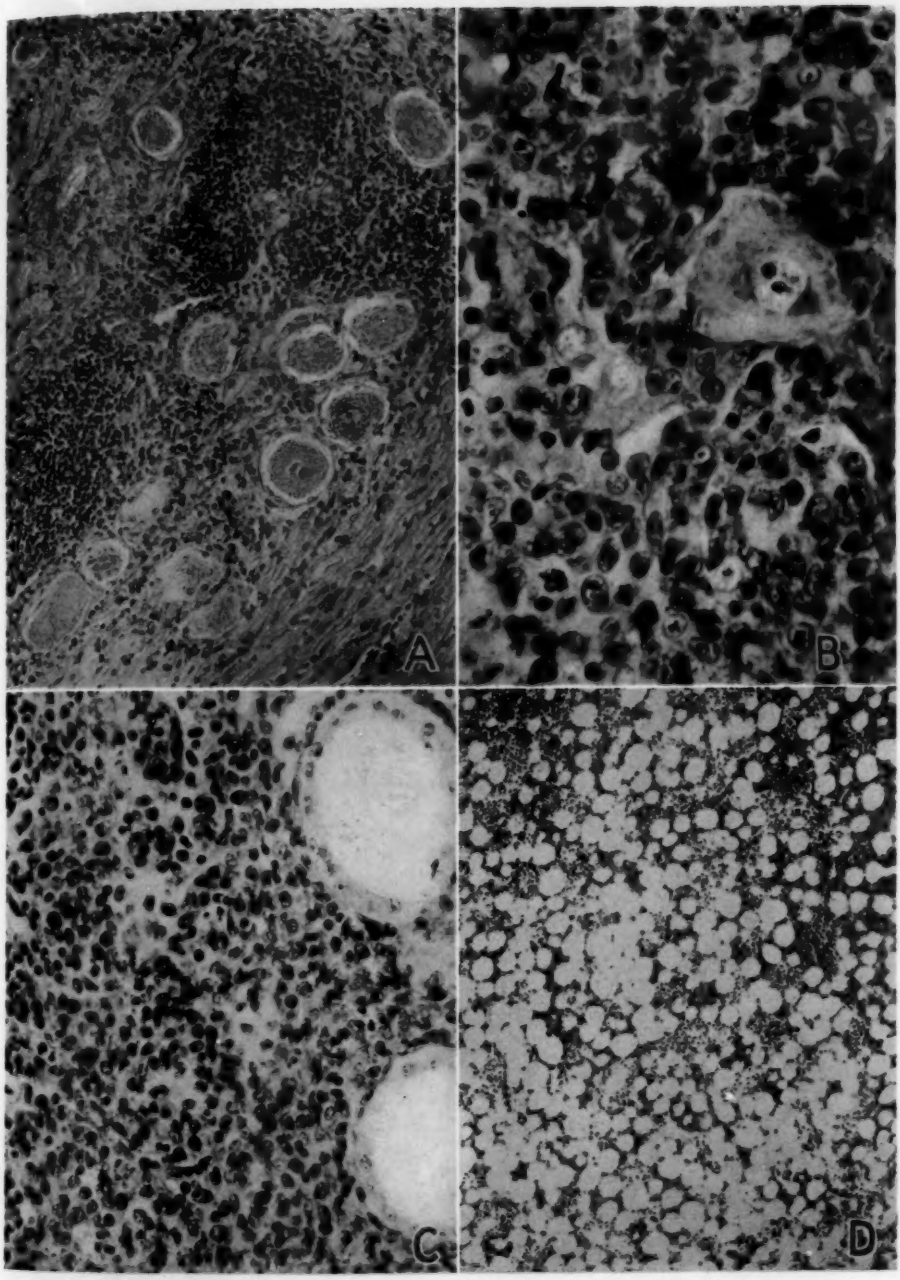

Fig. 11.—*A*, ganglion of a chicken with spontaneous neurolymphomatosis; the lymphoid infiltration does not appear to be neoplastic; × 120. *B*, ganglion of a chicken with transmitted lymphomatosis (strain 5), showing neoplastic infiltration; × 450. *C*, ovarian lymphoma (strain 5); × 280. *D*, the bone marrow of a chicken with spontaneous neurolymphomatosis showing no evidence of hyperplasia or lymphoid infiltration; × 60. The sections were stained with eosin and azure II solutions. The magnifications given are approximate.

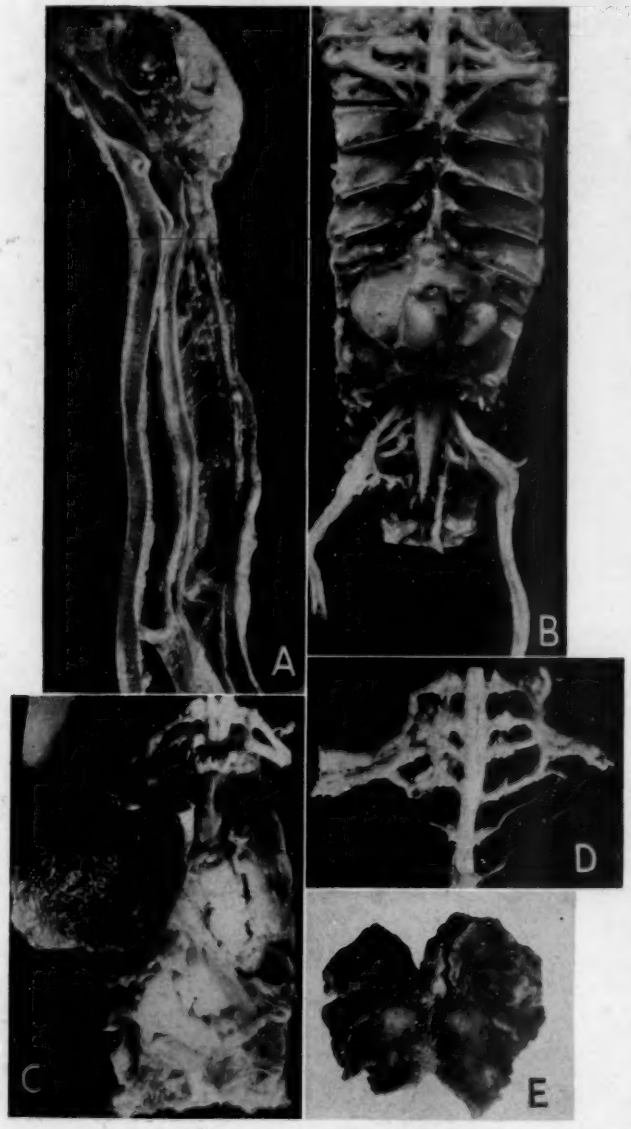

Fig. 12.—*A*, lymphomatosis of the vagus (strain 5). *B*, lymphoma infiltrating the adrenal, adjacent ganglions and upper lobe of the right kidney. Lymphomatosis of the sciatic nerves and brachial plexuses. *C*, lymphoma of the ovary infiltrating the upper lobes of both kidneys and adjacent parts of the right lung. *D*, lymphomatosis of the brachial plexuses and ganglions. *E*, lymphomatosis of the lung. The last four pictures represent chickens with spontaneous neurolymphomatosis.

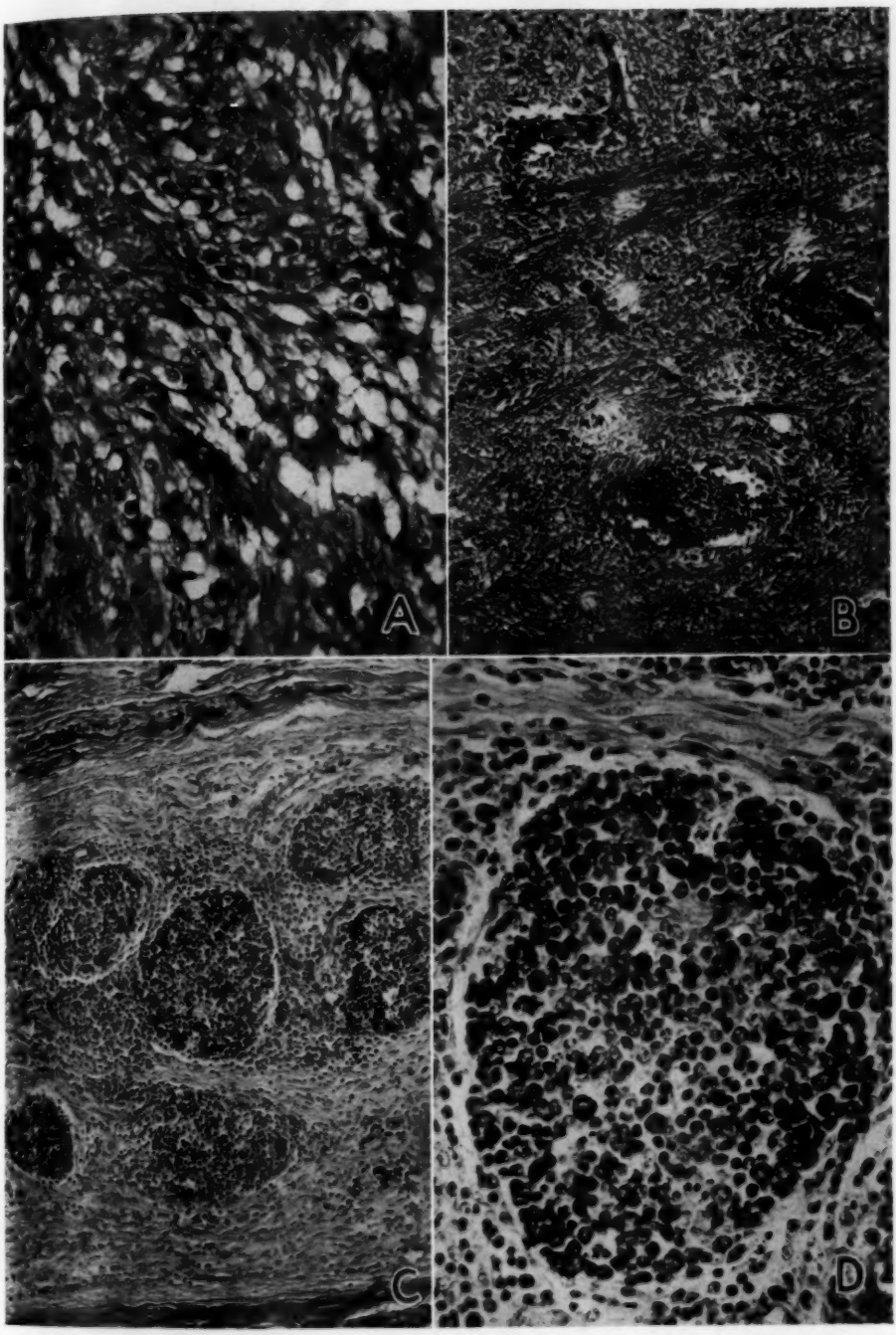

Fig. 13.—*A*, microscopic structure of a tumor of the brain found in a chicken that received an injection of tissues from paralyzed birds (strain 5); × 350. *B*, perivascular small round cell infiltrations in the brain of a chicken that received an injection of strain 2; × 100. *C* and *D*, lymphoid tissue with germinal centers in the sciatic nerve of a chicken at the site of intraneural injection of mouse brain carrying the virus of lymphogranuloma inguinale. The sections were stained with eosin and azure II solutions. The magnifications are approximately: *C*, × 100; *D*, × 300.

The spinal and sympathetic ganglions are favorite sites of infiltrations that are continuous with those of the peripheral nerves.

Perivascular round cell infiltration in the brain and cerebellum (fig. 13 *B*) was seen in numerous birds, but it is doubtful whether this infiltration was caused by the agent of neurolymphomatosis, for the following reasons: (*a*) The infiltration in the brain was composed of small lymphocytes and occasional cells like monocytes, and mitosis among these cells was scant even in chickens in which the coexistent infiltration of peripheral nerves and viscera was composed of lymphocytes of medium size with abundant mitotic figures; (*b*) similar alterations in the brain are very often found in apparently normal chickens (Pappenheimer, Dunn and Seidlin); (*c*) these alterations have the characteristics of encephalitis, not those of a neoplasm.

The cord was often normal even in the presence of extensive infiltration of the adjacent spinal ganglions. Less often it showed mild, occasionally extensive infiltration, which was usually perivascular and continuous with the meningeal and neural infiltration. It is possible that in these cases the lymphoid cells reached the cord by way of the peripheral nerves.

In one chicken (3862) a spherical tumor about 0.8 cm. in diameter was found in the cerebrum. The microscopic appearance of this tumor, shown in figure 13 *A*, resembled that of a spongioblastoma.[22]

Infiltration amounting to tumor formation about the inoculated sciatic nerve is shown in figure 10 *A*. The diagnosis of incipient neurolymphomatosis of the inoculated nerves offered great difficulties because of the nonspecific changes caused by the traumatism. The formation of lymphoid follicles about the nerve as shown in figure 13 *C* and *D* was considered a nonspecific alteration.

Among the viscera, the ovary was the most frequent site of infiltration (figs. 11 *C* and 12 *C*). It was enlarged to several times its normal size; its normal lobulated structure was obliterated by the yellowish-gray, soft, infiltrating lymphomatous tissue. In two instances, however, neurolymphomatosis was found in apparently healthy egg-laying chickens. The behavior of the blood-forming organs, bone marrow (fig. 11 *D*), spleen and liver (fig. 5 *C*) is noteworthy. In the majority of cases they showed no conspicuous alteration. The lymphomatous infiltration occasionally found in the bone marrow was nodular and replaced only a small part of the marrow; in the liver (fig. 5 *C*) the infiltrations were periportal; in the spleen they were found both in the pulp and in the follicles. The normal structure of these organs was, however, invariably retained. Lymphomatous infiltration in the kidney

22. Dr. Lewis D. Stevenson examined sections of the central nervous system and interpreted the lesions found.

and adrenal are shown in figure 12 *B* and *C*. The right lung in figure 12 *C* is normal, whereas that of figure 12 *E* is infiltrated by malignant lymphocytes.

TRANSMISSION WITH STRAIN 5

Origin of Strain.—Twenty-three chickens with clinical symptoms of neurolymphomatosis, found in the state of Delaware, were killed after a period of observation of from one to thirteen days. Postmortem examination of thirteen of these chickens showed thickening of nerves with no tumor formation; another eight showed thickening of nerves and gross visceral infiltration or tumors, and in two there was no gross or microscopic evidence of neurolymphomatosis.

It is evident that neurolymphomatosis can be diagnosed fairly accurately by clinical examination since only two of the twenty-three chickens received failed to show gross evidence of the disease. These two chickens have not been studied microscopically. Most of these chickens, including the two that showed no gross evidence of neurolymphomatosis, were infested with tapeworms.

Unsuccessful inoculations were made in twelve chickens, each receiving one or more injections of blood or nerve emulsion from one or more chickens with spontaneous paralysis.

The first successful inoculations were made from a chicken (3878) with spontaneous neurolymphomatosis, and the transmissible strain derived from this fowl is called strain 5.

Spontaneous neurolymphomatosis in this chicken was associated with lymphomatosis of the viscera and lymphatic leukemia. The association of lymphatic leukemia with neurolymphomatosis was hitherto unknown.

The bird was a much emaciated pale young Barred Rock hen that weighed 600 Gm. Its erythrocyte count was 2,950,000; the leukocyte count, 145,000; 92 per cent of the leukocytes were lymphocytes, mostly of small size (fig. 9 *B* and *C*). The hematocrit values of the blood were: erythrocytes, 33.5; leukocytes, 4.5; plasma, 62. The relatively low hematocrit value for leukocytes was due to the small size of the lymphocytes.

Both the legs and the wings were weak or paralyzed, the right more so than the left. At autopsy the vagus nerves, the brachial plexuses and their branches, and the lumbosacral plexuses and their branches were greatly thickened. The left brachial plexus was estimated to have a diameter of from three to five times normal, and attached to one of its branches there was a gray tumor nodule measuring 0.7 cm. across. About one third of the left lung was the site of gray lymphomatous infiltration. In the skin of the neck there was a lymphoma about 2.5 cm. in diameter, and a smaller lymphoma was found in the skin of the left leg. Surrounding the left carotid artery there was a fusiform tumor 0.5 cm. across. There were gray spots in the voluntary muscles, and microscopic examination of these areas showed extensive interstitial infiltration with lymphocytes mostly of medium size, many in mitosis. The bone marrow was normal and contained abundant fat. Grossly, the liver appeared normal; the spleen was slightly enlarged, and its follicles were prominent.

The lymphocytes that caused the extensive infiltration of nerves and viscera and invaded the blood stream were mainly of medium and small size, indistinguishable morphologically from normal lymphocytes. The alterations found in the tissues were indistinguishable from lymphomatous neoplasms of mammals.

Experiments.—Six chickens weighing from 850 to 1,100 Gm. were given intravenous injections of 4 cc. of the blood of chicken 3878. The inoculations were successful with one exception (table 4). This chicken (3849) has since been twice reinoculated intravenously with tissues derived from paralyzed chickens, without ill effects.

The second subpassages were also made by intravenous injections of large amounts of blood (from 1 to 10 cc.) and, with the exception

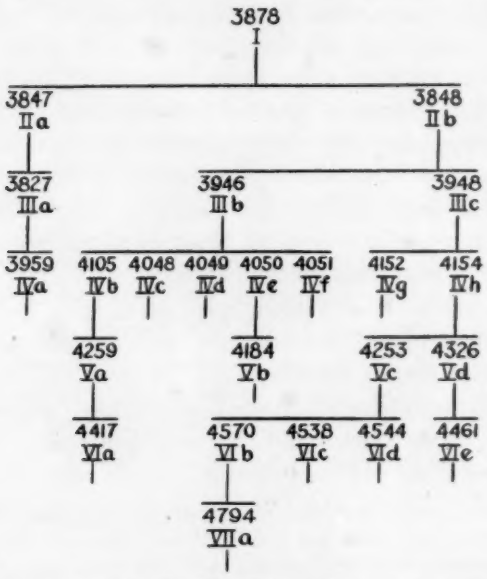

Fig. 14.—Passages of strain 5. The Roman figures show the number of the passage; the Arabic figures, the number of the chicken from which the passage was made. The results of inoculation are summarized in table 4.

of two chickens that died of early intercurrent infection of the upper respiratory passages, all inoculated chickens acquired neurolymphomatosis. The results of these and twenty subsequent passages are summarized in figure 14 and in table 4.

TRANSMISSION WITH STRAIN 6

Passages made with strain 6 neurolymphomatosis are shown in figure 15 and in table 5. The origin of this strain is somewhat obscure.

A chicken (3850) weighing 950 Gm. received intravenously 21 cc. of blood from two chickens with spontaneous neurolymphomatosis, and

TABLE 4.—*Transmission of Neurolymphomatosis (Strain 5) with Cell-Containing Material*

Passage	Chickens Inoculated	Successful Inoculations	Material Injected	Route of Injection*	Length of Life and Results of Injection†
I	6	4	Blood	Intrav.	K8+, K32+, D36+, D40—, D46+, alive —
IIa	4	2	Blood	Intrav.	D34—, D51—, K59+, K78+
IIb	4	4	Blood	Intrav.	K43+, K49+, K75+, K77+
IIIa	3	1	Blood	Intrav.	D36—, D48—, K84+
IIIb	7	6	Blood	Intrav.	K24+, K41+, K66+, K77+, K97+, + recovered, alive —
IIIc 12/31	3	1	Blood	Intrav.	D55—, K55+, K73—
1/ 7	4	4	Blood	Intrav.	K36+, K51+, K56+, K71+
IVa 11/23	4	1	Blood	Intrav.	K64+, alive —, alive —, alive —
11/27	4	3	Tumor	Intram.	D68+, D68+, K72+, alive —
IVb 12/ 9	4	1	Blood	Intrav.	D40—, K45+, D72+
12/18	4	4	Blood and spleen Tumor	Intrav. Intram.	D44+, K54+, K70+. K123+
IVc	3	2	Blood	Intrav.	D48±, K48+, D66+
IVd	6	2	Blood	Intrav.	K54+, K77—, D101+, alive —, alive —, alive —
IVe 11/23	4	2	Blood	Intrav.	K37—, K37—, K37+, K155+
11/29	3	2	Tumor	Intram.	K66+, K105+, alive —
IVf	1	1	Blood	Intrav.	K65+
IVg	3	0	Blood	Intrav.	D51—, K108—, alive —
IVh	4	3	Blood	Intrav.	D53+, K65+, K96+, K99—
Va	3	1	Blood	Intrav.	K68+, D98—, alive —
Vb	3	1	Blood	Intrav.	K59+, D90±, alive —
Ve	7	4	Blood	Intrav.	K40—, K43+, K49+, D56+, D61±, K104+, K162—
	4	2	Blood	Intran.	D33—, D34+, K38—, D51+
Vd	3	2	Blood	Intrav.	K58+, K79+, K112—
	3	2	Blood	Intran.	D29+, D48+, K112—
VIa	2	1	Tumor	Intran.	D25+, K145—
VIb	3	2	Blood and tumor	Intran.	K36+, K60+, K123—
VIc	2	2	Blood	Intran.	D34+, D43+
VId	3	0	Blood	Intrav.	K144—, K144—, K144—
VIe	3	1	Tumor	Intran.	D39—, D60+, K145±
VIIa	3	0	Blood and nerve	Intran.	D22±, K124—, K124—
Total	110	61			

* The abbreviation Intrav. means intravenous; Intram., intramuscular; Intran., intraneural.

† K signifies that the chicken was killed; D, that the chicken died. The numerals state the length of life. The plus and minus signs indicate that neurolymphomatosis was (+) or was not (—) found on examination, or that the success of the inoculation was doubtful (±). Thus K8+ means that a chicken killed thirty-two days after inoculation showed evidence of neurolymphomatosis. "Alive —" means that the chicken was unaffected after a period of from four to five months. Most of the chickens designated in the tables as "alive" were used for studies of immunity in relation to fowl paralysis.

five days later it was inoculated with a suspension of cells of a lymphomatous tumor of a paralyzed chicken. The purpose of this experiment was to shorten the period of incubation and to overcome the resistance of the host by massive doses. Six days after the inoculation the chicken showed conspicuous weakness of the left leg and left wing, and two healthy chickens were each given an injection of 5 cc. of its blood. Both these chickens died with neurolymphomatosis (table 5), but chicken 3850, on which autopsy was done three days after these inoculations were made, showed peritonitis and pneumonia with no gross or microscopic evidence of neurolymphomatosis. Nevertheless, it carried the agent of neurolymphomatosis.

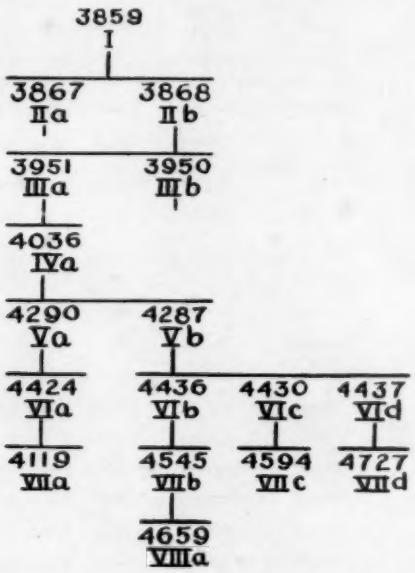

Fig. 15.—Passages of strain 6. The Roman figures show the number of the passage; the Arabic figures, the number of the chicken from which the passage was made. The results are summarized in table 5.

Three of the four chickens of the first subpassage (IIa of table 5) were lost in an epidemic of fowl pox and were discarded without microscopic study. One chicken of the second passage (IIb) presented paralysis, but four chickens (passage IIIb) inoculated with its blood were unaffected; another chicken of the second passage showed leukemic myelocytomatosis with no evidence of neurolymphomatosis. Since this was the only instance of leukosis other than lymphomatosis observed in this study involving approximately three hundred chickens, no significance can be attached to its occurrence. This chicken was probably a carrier of the agent of neurolymphomatosis, for one of the three

chickens inoculated with its blood (4036, passage IIIa) acquired typical neurolymphomatosis. Chicken 4036 is one of the three chickens designated in figure 15 and in tables 4 and 5 as successfully inoculated because its blood contained the transmitting agent in spite of the absence of the anatomic changes of neurolymphomatosis.

TABLE 5.—*Transmission of Neurolymphomatosis (Strain 6) with Cell-Containing Material*

Passage	Chickens Inoculated	Successful Inoculations	Material Injected	Route of Injection*	Length of Life and Results of Injection*
I	2	2	Blood	Intrav.	K49+, K59+
IIa	4	0	Blood	Intrav.	K20—, K25—, K25—, alive —
IIb	4	2	Blood	Intrav.	K23+, K39+, alive —, alive —
IIIa	3	1	Blood	Intrav.	K76+, K77—, K77—
IIIb	4	0	Blood	Intrav.	Alive —, alive —, alive —, alive —
IVa 12/16	3	0	Blood	Intrav.	D25—, D30—, alive —
12/19	6	4	Blood or nerve	Intrav. or intran.	K38+, K53+, D44+, K56+, K78—, alive —
Va	3	3	Blood or nerve	Intran.	K33+, K56+, K65+
Vb	9	8	Blood or nerve	Intrav. or intran.	K40+, D47+, D53+, K54+, K62+, D95—, D97+, K111+, K117+
VIa	3	1	Nerve	Intran.	K32+, K48±, K154—
VIb	5	3	Blood	Intrav. or intran.	K41+, K50+, D55+, K66—, K114—
VIc	2	2	Nerve	Intran.	D44+, D62+
VId	4	3	Blood	Intrav.	D49+, K56+, K82+, K156—
VIIa	3	1	Blood or nerve	Intran.	K88+, K102—, K116—
VIIb	3	3	Blood or nerve	Intram. and intran.	K36+, K51+, K122+
VIIc	3	0	Blood	Intrav and intran.	K124—, K124—, K124—
VIId	3	1	Blood or tumor	Intran.	D20—, D24+, K120—
VIIIa	2	1	Blood	Intran.	K55+, D90—
Totals	66	35			

* The abbreviations are explained in the footnote to table 4.

The history of further subpassages is shown in table 5. The introduction of intraneural injection has greatly increased the percentage of successful inoculations, and the extensive infiltration in and about the nerve receiving the injection has revealed the success of the injection shortly after inoculation.

PRESENCE OF THE TRANSMITTING AGENT IN THE BLOOD

The first question that confronted us was whether transfers with blood should be made at the early or at the late stage of the disease. It is known that in most common virus diseases the appearance of

antibodies is followed by disappearance of the virus from the blood, but leukosis is readily transmitted with blood at an advanced stage of the disease. Quantitative studies on the concentration of the transmitting agent of leukosis and of Rous sarcoma in the blood have thus far, to our knowledge, not been made, but our experience with leukosis indicates that the disease is transmissible with the blood of leukotic chickens at all stages of illness. The results of an experiment made with strain 5 are recorded in table 6. The chicken whose blood was used in this experiment was inoculated on September 1 and showed the first signs of paralysis on October 13. Tests for the presence of the transmitting agent in its blood were made on October 13, October 31 and November 13. Two days later, when moribund, the chicken was killed. It showed extensive thickening of numerous nerves but no infiltration or tumors in the viscera.

TABLE 6.—*Data on Transfers of Strain 5 by Injections of Blood at Successive Stages of Illness*

Date of Injection	Result with 0.5 Cc.	Result with 5 Cc.
October 13	K24+	K41+, alive —
October 13 and 31	+ recovered	K77+
October 31	K66+	K97+
November 13	D36+, D54+	

Similar experiments were made with the blood of a chicken that was inoculated on September 1 and showed the first signs of paralysis on October 31. The first inoculation, made on October 31, was much less successful than the second, made with blood taken from the heart immediately after death (November 17): Inoculations on October 31 with 0.5, 2 and 10 cc. of blood gave, respectively, negative results in a chicken that died at 55 days, neurolymphomatosis in one that was killed at 55 days and negative results in one that was killed at 73 days. Inoculations on November 17 with 0.2 cc. of blood gave neurolymphomatosis in chickens killed at thirty-six, fifty-one, fifty-six and seventy-one days, respectively, after inoculation.

These experiments show that the transmitting agent circulates in the blood of the paralyzed chicken during the entire period of manifest disease. Economic reasons prevented us from determining accurately the concentration of the transmitting agent in the blood. In subsequent work, however, the transfers were successfully made, as with leukosis, from chickens with advanced disease. Nevertheless, quantitative comparative determination of the presence of oncogenic agents and of common viruses in the blood at various stages of illness is desirable.

Concentration of the Transmitting Agent in the Blood.—Intraneural inoculations were made with approximately from 0.01 to 0.02 cc. of

blood, and since the majority of the chickens thus inoculated presented the disease within a comparatively short period of time, it is probable that the minimal infecting dose is much smaller.

Before the discovery of the efficiency of the intraneural route, attempts were made to determine the amount of blood required to transmit paralysis by the intravenous route. The results of such an experiment were as follows: With 1 cc., two chickens killed at forty-nine and one hundred and four days after inoculation showed neurolymphomatosis. With 0.01 cc., one chicken that died at fifty-six days presented neurolymphomatosis, while in another that died at sixty-one days the success of the inoculation was doubtful. With 0.0001 cc., one chicken that was killed at forty-three days showed neurolymphomatosis, while two that died at forty and one hundred and sixty-two days did not show any evidence of the disease.

TABLE 7.—*Experimental Study of Concentration of Transmitting Agent in Blood*

Experiment	Amount of Blood Injected, Cc.	Results of Injection*	Experiment	Amount of Blood Injected, Cc.	Results of Injection*
1	1	K75+	4	0.02	D101+, alive —
	2	K43+		0.2	K54+, K166—
	3	K49+		2	K77—, alive —
	10	D77+			
2	0.5	+ recovered, K24+, K66+,	5	0.5	Alive —, alive —
				5	K64+, alive —
	5	K77+, K41+, K97+, alive —	6	0.5	K37+
				5	K155+
3	0.5	D55—	7	0.02	D48+
	2	K55+		0.2	D66+
	10	K73—		2	K48+

* The abbreviations are explained in the footnote to table 4.

Preceding this experiment from 0.02 to 10 cc. of blood was used for intravenous inoculations, but the results appeared to be independent of the amount of blood injected, as shown in table 7.

INTRANEURAL TRANSMISSION

Failure of previous workers to increase the percentage of successful inoculations by intraneural injections or to cause local infiltration of the nerves receiving injections at first discouraged attempts to transmit the disease by this route, but when it was recognized that neurolymphomatosis is allied to Rous sarcoma and Ellermann's leukosis, these failures seemed paradoxical. For if the lymphocyte is the neoplastic cell in neurolymphomatosis and the peripheral nerves and ganglions are the common sites of infiltration, intraneural introduction of the malignant cells should bring about infiltration of the nerves receiving injections. Comparative intravenous and intraneural inoculations with the free virus, on the other hand, might determine whether the virus has a

special affinity for the nerves, which are secondarily invaded by lymphocytes, or for lymphocytes, which it renders malignant and endows with special affinity for the nerves.

The experiments recorded in table 8 show that with the blood of paralyzed chickens intraneural inoculations are more often successful than intravenous inoculations.

TABLE 8.—*Comparison of Intraneural with Intravenous Transfer*

Route of Injection	Results of Inoculation
Intravenous	K58+, K79+, K112—
Intraneural	D29+, D48+, alive —
Intravenous	D56+, D61—
Intraneural	D83—, D38—, D51+, D84+
Intravenous	K66—, K114—
Intraneural	K41+, K50+, D55+

Totals	Chickens Inoculated	Chickens with Neurolymphomatosis
Intravenous	7	3
Intraneural	10	7

TABLE 9.—*Comparison of Emulsion of Infiltrated Nerves with Whole Blood as a Medium of Transfer*

Material Injected	Amount Injected, Cc.	Route of Injection	Results of Injection
Blood	0.5	Intrav.	K54+, D95—, K97+
Nerve*	0.01	Intrav.	K40+, K110+, K117+
Nerve*	0.01	Intran.	D47+, D63+, K62+
Blood	1	Intrav.	K78—, K115—
Nerve*	0.01	Intran.	K38+, K53+
Nerve*	0.01	Intran. and intrav.	D44+, K56+
Blood	0.1		

Totals	Chickens Inoculated	Chickens with Neurolymphomatosis
Intravenous	8	5
Intraneural	7	7

* Suspension of infiltrated nerves.

An emulsion of infiltrated nerves transmits the disease as well as, if not better than, whole blood (table 9).

In the last two chickens referred to in table 9 an emulsion of infiltrated nerves was injected into one sciatic nerve and blood into the other. The nerve inoculated with emulsion of nerve cells was more thickened than that inoculated with blood. Nerve tissue itself is not required for transmission of the disease, for emulsions of lymphomatous tumors of the ovary and skin as well as blood readily transmit the disease.

In a later experiment the blood of a paralyzed chicken was injected into the right sciatic nerve and the blood of a normal chicken into the left sciatic nerve in two chickens. One remained healthy; the other, when killed fifty-five days after these injections, showed extensive thickening of both sciatic nerves associated with infiltration of several other nerves. This observation raised doubt concerning the feasibility of testing different materials by multiple intraneural injections into one chicken. Numerous control intraneural inoculations have shown that neurolymphomatosis can be produced only with tissues of lymphomatous chickens (see p. 38). It is possible that in birds carrying the virus of neurolymphomatosis localization of lymphomatous infiltration is facilitated by trauma. Such is the case with the virus of yellow fever.[23]

The following observations suggest that the blood stream is the major channel through which the agent spreads from one nerve to distant nerves:

(*a*) The inoculation into the sciatic nerve was made midway between the sciatic foramen and the popliteal fossa. The infiltration was extensive distal from the site of inoculation but was slight proximal to it.

(*b*) The spinal cord in neurolymphomatosis shows slight or no infiltration. There is only slight interstitial or perivascular infiltration in the cord even in instances in which the spinal ganglions are so extensively infiltrated that their structure is barely recognizable.

(*c*) The distant lesions after intraneural injection are as varied as after intravenous injection, and nerve tissue between the diseased and the inoculated nerve often appears normal.

(*d*) The blood contains the transmitting agent in high concentration and reproduces a disease indistinguishable from spontaneous neurolymphomatosis.

These observations are in accord with those summarized by Abel,[24] according to which the bulk of the material injected into and about nerves does not follow nerve routes but passes into the subclavian vein by way of the endoneural and perineural lymphatics.

The vasa lymphacea ischiadica of chickens accompany the sciatic nerve in the distal two thirds of the thigh (Baum[25]) and, passing along the median side of the os femoris, enter the peritoneal cavity and end in the thoracic duct.

Here the objection may be raised that both lymphocytes and virus might travel along the nerve fibers without causing anatomic alterations.

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23. Sawyer, W. A., and Lloyd, W.: J. Exper. Med. **54**:533, 1931.
24. Abel, J. J.: Science **79**:121, 1934.
25. Baum, H.: Ztschr. f. Anat. u. Entwcklngsgesch. **93**:1, 1930

Ease of transmission with cell-containing material and difficulty of transmission with injured cells suggest that the major factors in the spread of the disease are lymphocytes. The ease with which the disease is transmitted by blood suggests that the lymphocytes reach distant nerves by way of the blood stream. That infiltration extends along the inoculated nerve is certain, for the sciatic nerve becomes uniformly thickened distally from the site of injection; proximally, too, the infiltrations may extend as far as the cord. In one instance, in which the ulnar nerve was inoculated, it was uniformly thickened throughout its course. Thus some lymphocytes introduced in peripheral nerves may reach the meninges, as do brominized oil,[26] prussian blue [27] and some neurotropic viruses,[28] but systemic neurolymphomatosis appears to be the result of intravenous dissemination of the causative agent.

CONTROL INTRANEURAL INJECTIONS

The specificity of the alterations produced by intraneural injections of lymphomatosis strains 2, 5 and 6 was tested as follows:

Leukosis strain 1 produces erythroleukosis and myeloblastic leukemia. Its characteristics remained unchanged during five years of observation. The results of injections made with the blood of chickens with myeloid leukemia associated with erythroleukosis were as follows:

Experiment 1.—Intravenous injections gave myeloid leukemia in one chicken that died at thirty-nine days, and erythroleukosis in four chickens, three of which died at forty, forty-seven and fifty-one days, respectively, and one of which recovered. Intraneural injections gave erythroleukosis in two chickens that died at sixty-two and seventy days; myeloid leukemia and erythroleukosis in one that died at sixty-nine days; myeloid leukemia in one that died at seventy-four days, and no evidence of disease in one that was killed at ninety-one days.

Experiment 2.—Intravenous injections yielded negative results in two chickens killed at twenty-five and one hundred and three days and erythroleukosis in one killed at thirty-five days. Intraneural injections yielded erythroleukosis in a chicken that died at thirty-nine days and negative results in two chickens killed at one hundred and three days.

There was only a slight thickening of the nerves, similar to that caused by normal blood, at the site of puncture with the needle.

These experiments show that leukosis strain 1 is readily transmitted by intraneural inoculation, but it does not produce neurolymphomatosis.

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26. Sullivan, W. E., and Mortensen, O. A.: Anat. Rec. **59**:493, 1934.

27. Clark, W. E. LeG.: Report to Committee on Vaccination on an Anatomical Investigation into Routes by Which Infection May Pass from Nasal Cavities into Brain. Reports on Public Health and Medical Subject, no. 54, Ministry of Health, London, His Majesty's Stationery Office, 1929.

28. Hurst, E. W.: J. Path. & Bact. **33**:1133, 1930; J. Exper. Med. **59**:729, 1934.

Eight chickens given injections of the blood of normal chickens remained healthy.

In association with Dr. A. Grace, I inoculated three chickens intraneurally with the brain tissue of a mouse carrying the neurotropic virus of lymphogranuloma inguinale.[29] These chickens, killed twenty, fifty and seventy days after inoculation, showed at the site of inoculation formation of lymphoid tissue with germinal centers (fig. 13 *C* and *D*). Aside from this there was no lesion suggestive of lymphomatosis. One chicken inoculated intraneurally with a cell suspension of transmissible lymphomatosis of mice gave no evidence of neurolymphomatosis.

Summary.—Intraneural inoculations are followed by neurolymphomatosis only when the material injected is obtained from chickens with lymphomatosis.

RELATION OF LYMPHOMATOSIS OF VISCERA TO LYMPHOMATOSIS OF NERVES

The association of neurolymphomatosis with lymphomatous infiltration or lymphomas of the viscera has been noted by most of those who have worked with this disease, and observations made by Pappenheimer, Dunn and Cone [5] and several other workers suggest that both are caused by the same agent. This view has been contradicted by Mathews,[8b] who found that lymphomatosis is often unassociated with infiltration of the nerves. This is true for a common variety of lymphomatosis that is characterized by great enlargement of the liver ("big liver" disease, hepatolymphomatosis).

Experiments were made to determine the relationship of lyphomatous tumors occurring among the passages of strain 5 to the infiltration of the nervous system. In experiment 1 a suspension of infiltrated nerves produced extensive lymphomatous infiltration of the inoculated nerve, but it did not produce infiltration or tumors in the inoculated breast muscle. Experiments 2, 3 and 4 showed that the tissue of lymphomatous tumors occurring among the passages of strains 5 and 6, unlike strain 2 or lymphomas of mice, does not produce infiltration in the inoculated breast muscle, but may produce neurolymphomatosis unassociated with infiltration of the viscera.

Experiment 1.—An emulsion of infiltrated nerves (strain 5) was injected into both the right pectoral muscle and the right sciatic nerve in three chickens, and blood was injected into the left pectoral muscle and the left sciatic nerve in the same chickens. The birds were killed thirty-six, fifty-one and one hundred and twenty-two days after the injections were made. The sites of the intramuscular injections could not be detected, but all nerves that received injections were much thickened, those inoculated with nerve emulsion to a greater extent than those inoculated with blood.

29. Grace, A.: Proc. Soc. Exper. Biol. & Med. **32**:71, 1934.

Experiment 2.—Small pieces from a lymphomatous tumor (strain 5), about 8 by 6 by 2 cm., which had infiltrated the skin and muscles of the wing of a paralyzed chicken, were injected into the muscles and subcutaneous tissue of the breast and leg in four chickens. One of these chickens remained healthy; the second became paralyzed and died sixty-eight days after inoculation with gross evidence of extensive neurolymphomatosis unassociated with lymphomatosis of other organs. The third, killed sixty-eight days after injection because of fowl pox, showed on microscopic examination conspicuous lymphoid infiltrations of nerves and of the sympathetic ganglion about the adrenal. The fourth, killed seventy-two days after injection because of roup, showed on microscopic examination distinct lymphoid infiltration of several nerves. None of these chickens showed infiltration at the sites of the injections.

Experiment 3.—Lymphomatous tumor tissue from a paralyzed chicken (strain 5) was injected into the pectoral muscles of three chickens. None of these chickens had infiltration in the inoculated muscles. One remained healthy, the second presented characteristic paralysis with no tumors, and the third showed diffuse lymphomatosis.

Experiment 4.—In this experiment four chickens were inoculated by way of the pectoral muscle with tumor tissue of a paralyzed chicken (strain 5). All four acquired neurolymphomatosis unassociated with lymphomatous infiltration of the inoculated muscle or of the viscera. Since each of these chickens also received an intravenous injection of blood, the general disease may have been produced with blood.

In the following experiments lymphomatous tumor tissue occurring in association with neurolymphomatosis was injected into the sciatic nerves of healthy chickens, and it produced typical neurolymphomatosis unassociated with lymphomatosis of the viscera.

Experiment 5.—Tissue of an ovarian lymphoma occurring in a paralyzed chicken (strain 5) was injected into the sciatic nerve in three chicks. One that died thirty-nine days after inoculation and another that died sixty days after inoculation showed extensive lymphomatous infiltration of the inoculated nerves; the third remained healthy.

Experiment 6.—Tissue of a lymphoma of the breast muscle of a paralyzed chicken was injected into the sciatic nerve in two chickens. One of these died thirty-five days after inoculation with extensive lymphomatosis of the inoculated nerve and general neurolymphomatosis unassociated with tumor formation. The second chicken remained healthy.

Experiment 7.—Tissue of an ovarian lymphoma of a paralyzed chicken (strain 5) was injected into the left sciatic nerve and blood of the same paralyzed chicken was injected into the right sciatic nerve in three chicks. Two died from twenty to twenty-four days after inoculation, showing neurolymphomatosis, mainly of the inoculated nerves; the third remained healthy.

These experiments suggest that tumor tissue and suspensions of infiltrated nerves transmit neurolymphomatosis more readily than blood, and support the view that the lymphocytes that form tumors and infiltrate nerves are the cells responsible for the transmission of the disease. It may be assumed that transmission of the disease is due to a virus and that the lymphocytes are carriers of this virus, but the

experiment reviewed in the next section failed to demonstrate transmission by cell-free material.

The tumor tissue in all these experiments was derived from paralyzed chickens. Lymphomatosis of the viscera unassociated with lymphomatosis of the nervous system was found only on gross examination. In the following experiment, blood and tumor of a chicken that had extensive lymphomatous infiltration of the viscera, but only microscopic neural infiltrations, produced extensive neurolymphomatosis:

Experiment 8.—Chicken 4570 (strain 5) died ninety-two days after intraneural inoculation with blood frozen at —30 C. for thirty minutes. At autopsy, numerous lymphomas were found in the heart, lung and skin, the largest being about 1 cm. in the longest diameter. Microscopic examination showed moderate lymphomatosis of the peripheral nerves. The heart blood of this chicken was injected into the left sciatic nerve and tumor tissue into the right sciatic nerve in three chickens. Two of these, killed thirty-six and sixty days after inoculation, showed neurolymphomatosis with extensive infiltration of both inoculated nerves; the third remained healthy.

Summary.—Tissue from lymphomatous tumors occurring among passages of strains 5 and 6, when inoculated intraneurally or intramuscularly into healthy chickens, produced neurolymphomatosis with or without lymphomatosis of viscera. After intramuscular injection it failed to produce tumors in the inoculated muscles; nevertheless, it produced neurolymphomatosis. After intraneural injection it produced extensive lymphomatous infiltration of the inoculated nerves.

ATTEMPTS TO DEMONSTRATE CELL-FREE TRANSMISSION

Effect of Freezing and Thawing.—Freezing inactivated the transmitting agent in all but one experiment (the fourth of table 10).

Previous experiments have shown that blood cells are destroyed when exposed for thirty minutes to a temperature below approximately —15 C.,[30] but the microorganisms tested and the agents of chicken leukosis and sarcoma[31] are resistant to much lower temperatures. In the first six experiments of table 10 the material to be frozen was sealed in a test tube and submerged for thirty minutes in alcohol cooled with solid carbon dioxide to a temperature of from —25 to —30 C. In the last experiments an attempt was made to determine the subzero temperature at which the transmitting agent became inactivated. From 0.2 to 1 cc. of blood was injected intravenously and about 0.02 cc. intraneurally.

The meaning of these experiments is obscure. It is improbable that a virus can be destroyed by thirty minutes' exposure to a temperature of from 20 to 30 C. The possibility remains that the malignant lymphocytes contain a filtrable agent which is inactive in the absence of live cells, requiring their presence to obtain a foothold in a new host.

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30. Furth, J.; Seibold, H. R., and Rathbone, R. R.: Am. J. Cancer **19**:521, 1933. Furth, J.: J. Exper. Med. **61**:423, 1935.

31. (*a*) Stubbs, E. L., and Furth, J. J.: J. Exper. Med. **61**:593,1935. (*b*) Furth. [4a]

These experiments demonstrate the necessity of using live cells for transmission of the disease, and explain the unsuccessful attempts of those who have attempted transmission by crushing the cells with the purpose of obtaining the hypothetic virus.

TABLE 10.—*Effect of Freezing on the Transmitting Agent*

Material Injected	Route of Injection*	Results of Injection*
Fresh blood	Intrav.	D36+, D54+
Frozen blood	Intrav.	Alive —, alive —
Fresh blood	Intrav.	K68+, K99—, alive —
Frozen blood	Intrav.	D90—, K109—, K160—
Fresh blood	Intrav.	K49+, K104+
Frozen blood	Intrav.	D53—, K101—
Fresh blood	Intran.	D56+, D61±
Frozen blood	Intran.	K58+, D64—, D78—, D92+
Fresh blood	Intran.	K33+, K65+, D56+
Frozen blood	Intran.	K101—, K150—, K150—
Fresh nerve	Intran.	D16+, D44+, D62+
Frozen nerve	Intran.	D35—, D50—, K67—
Fresh blood	Intran.	D43+, D43+
Blood frozen at —10 and —15 C	Intran.	D19—, D89—, D90—
Blood frozen at —20 C	Intran.	K28—, D80—, K122—, K122—

Totals	Chickens Inoculated	Chickens with Neurolymphomatosis	Chickens in Which Result Was Doubtful	Chickens Unaffected
Fresh material	17	14	1	2
Frozen material	24	2	0	22

* The abbreviations are explained in the footnote to table 4.

TABLE 11.—*Results of Inoculations with Plasma*

Material Injected	Amount Injected, Cc.	Route of Injection*	Results of Injection*
Whole blood	0.5	Intrav.	D36+, D54+
Plasma	0.5	Intrav.	K130±, K202—
Blood cells	0.5	Intrav.	K129+, K202—
Whole blood	0.2	Intrav.	K96+, K99—
Plasma	0.2	Intrav.	K78—, K117+
Blood cells	0.2	Intrav.	D53+, K65+
Whole blood	0.02	Intrav.	K66—, K114—
Plasma	0.02	Intrav.	D30—, D62—, D66—
Plasma	1.0	Intrav.	K80—, K163—
Whole blood	0.02	Intran.	K41+, K50+, D55+
Plasma	0.02	Intran.	K34—, D84—, K113— (sarcoma)

* The abbreviations are explained in the footnote to table 4.

Attempts at Transmission with Plasma.—One of nine chickens inoculated intravenously with plasma presented paralysis; all of three chickens inoculated intraneurally remained healthy (table 11).

The plasma was obtained by spinning heparinized blood at approximately 1,000 revolutions per minute and recentrifugating the plasma at approximately 2,000 revolutions per minute for from twenty to thirty minutes. The plasma was carefully removed to avoid its contamination with cells. Previous experiments had shown that plasma obtained under these conditions was cell-free.

Attempts to transmit lymphomatosis and myelosis of mice with plasma similarly obtained had been uniformly unsuccessful.[30] Failure of intraneural inoculations with cell-free material other than plasma made in a large number of chicks will be described in the next section. The sarcoma that appeared in the lumbar region in one of the chickens inoculated with plasma proved to be transmissible by tumor filtrates and desiccates, but its virus did not produce paralysis (strain 15).

TABLE 12.—*Effect of Drying on the Transmitting Agent*

Material Injected	Route of Injection*	Age of Dried Material, Days	Results of Inoculation*
Fresh blood	Intrav.	—	D31+, D54+, K129+, K200—
Dried blood	Intrav.	86	K120—, K163±, K163—, K163—
Fresh blood	Intrav.	—	K25+, K43+, K49+, D77+
Dried blood	Intrav.	159	K36—, D40—, K163—
Fresh blood	Intrav.	—	D44+, K54+, K70+, K123+
Dried blood	Intrav.	61	K99+, K163—, K163—
Mixture of 3 dried samples	Intrav.	61 to 150	D54—, K163—, K163—
Fresh nerve	Intran.	—	D16±, D44+, D62+
Dried nerve and blood	Intran. and intrav.	1	D33—, D41—, D43—, D45—, D101—, K148—
Dried blood	Intran.	1	D45—, K148—, K148—

Totals	Experiments	Chickens Inoculated	Successful Injections
Fresh (control) material	4	15	13
Dried material	6	22	1

* The abbreviations are explained in the footnote to table 4.

Attempts at Transmission with Desiccated Tissues.—Table 12 is a summary of unsuccessful attempts to preserve by drying the transmitting agents of strains 5 and 6. Only one of twenty-two chickens given injections of dried tissues of paralyzed birds died with paralysis.

Rous and Murphy [32] found that some agents of sarcoma can be preserved by drying, whereas others cannot. Drying was usually successful with our leukosis strain 1,[33] sarcoma strains 11 and 15 [34] and sarcoma-leukosis strain 13 (Stubbs and Furth [31a]), often successful with leukosis strain 2 [35] and occasionally successful with osteochrondrosarcoma-leukosis strain 12.[34]

32. Rous, P., and Murphy, James: J. Exper. Med. **19**:52, 1914.
33. Furth, J.: J. Exper. Med. **55**:495, 1932.
34. Furth, J.: Unpublished Work.
35. Furth.[4a, 34]

The technic of drying from the frozen state has been described.[4a] The dried material was stored in the icebox in sealed test tubes, and only after the corresponding fresh samples were found to be highly active were the stored samples used for the injections. Each chicken received approximately 50 mg. of dried blood taken up in Locke's solution. In the last two experiments dried blood was injected twenty-four hours after the injection of the fresh blood. In the first four experiments dried blood was injected intravenously; in the fifth experiment dried blood was injected intravenously and into one sciatic nerve, while the other sciatic nerve received an injection of dried suspension of an infiltrated nerve. In the sixth experiment dried blood was injected into one sciatic nerve and a suspension of infiltrated nerve was injected into the other sciatic nerve. The uniform results obtained after drying under identical conditions each of our different oncogenic strains makes it unlikely that failure to preserve the agents of neurolymphomatosis by drying is due to faulty technic.

Summary.—Neurolymphomatosis occurred in four of the fifty-eight chickens inoculated with material free from viable cells. The number of positive results is too small to be significant. If neurolymphomatosis is caused by a virus, the conditions under which this virus acts remain to be demonstrated.

TABLE 13.—*Summary of Attempts to Demonstrate Transmission of Neurolymphomatosis by Cell-Free Material*

Material Injected	Chickens Inoculated	Chickens with Neurolymphomatosis
Dried	24	1
Frozen	10	2
Plasma	24	1
Total	58	4 (7%)

III. CONTROL OBSERVATIONS REGARDING TRANSMISSIBILITY OF LYMPHOMATOSIS

The frequent occurrence of spontaneous neurolymphomatosis suggested a study of its incidence among our laboratory animals.

UNINOCULATED CHICKENS

Uninoculated chickens that could be regarded as controls were few in these studies, mainly because we assumed that each strain was well controlled by the rest of our flock. The fate of twenty-nine uninoculated chickens observed mainly during the first phase of these studies is, however, of some interest. The vertical line of figure 16 shows the number of chickens, the horizontal line the period during which they were observed in the laboratory; for example, after three months twenty-five of the twenty-nine chickens were alive and none of the four that had died had leukosis or sarcoma. Later three of the twenty-nine uninoculated control chickens died with aleukemic lymphomatosis, but none of them showed gross evidence of neurolymphomatosis; one

died with sarcoma. These as well as the similar figures given in previous reports urge caution in interpreting the results of transmission experiments made without controls and on small numbers of animals. Although paralysis was not seen in this series, one of forty young chickens ("broilers") received from the farmer who supplied us with most of our birds showed advanced paralysis four days after arrival.

The incidence of spontaneous neoplasms among these twenty-nine control chickens was unexpectedly high. The chickens used in studies of leukosis strains 1 and 2 were usually destroyed three months after inoculation. Only one instance of leukosis occurred among the twenty-

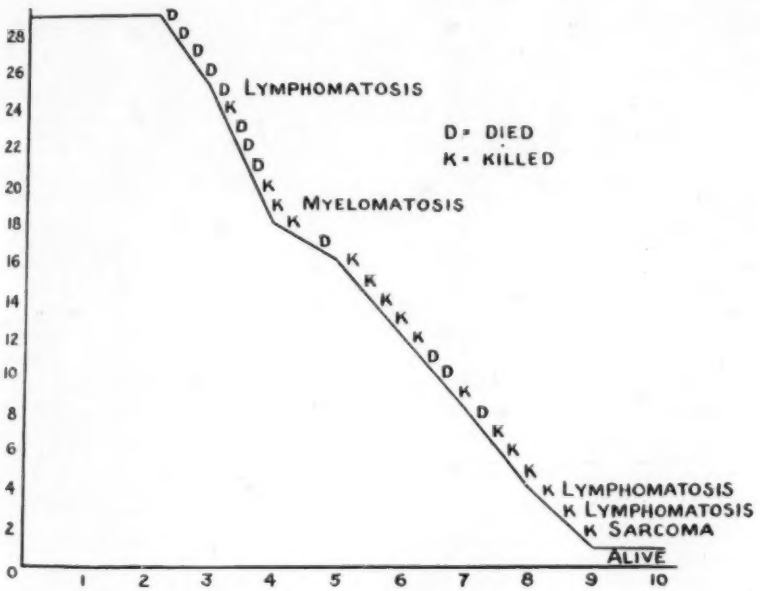

Fig. 16.—The number of the uninoculated control chickens in relation to the period of observation and to the spontaneous occurrence of lymphomatosis, myelomatosis and sarcoma. The vertical line of figures gives the numbers of chickens alive at the close of the periods of observation in months, shown in the horizontal line of figures. For example, after three months twenty-five of the twenty-nine chickens were alive and none of the four that had died (D) had leukosis or sarcoma.

nine control birds within this period of time. Experiments on neurolymphomatosis were terminated after approximately five months, and only one instance of lymphomatosis and one instance of myelocytomatosis were found among the uninoculated control chickens within this time. Many of the control chickens were killed after five months, but of the twelve chickens that were kept longer than six months two presented lymphomatosis and one sarcoma. We noted on previous

occasions the high incidence of similar neoplasms among chickens used in studies on active immunity to leukosis and kept in the laboratory during a period of from one to two years.

INCIDENCE OF NEUROLYMPHOMATOSIS AMONG CHICKENS INOCULATED WITH TRANSMISSIBLE STRAINS OF LEUKOSIS AND SARCOMA

These birds were kept in the same animal room that housed the chickens inoculated with tissues of paralyzed birds, but usually in different cages. From November 5 to April 27 a weekly record was kept

TABLE 14.—*A Periodical Record of the Incidence of Neurolymphomatosis Among the Chickens in Our Animal Rooms*

	Neurolymphomatosis Strains 5, 6, 4, 14		Leukosis Strain 2		All Other Strains of Leukosis and Sarcoma		Total Number of Chickens in Animal Room
Date	Chickens in Experiment	Incidence of Neurolymphomatosis	Chickens in Experiment	Incidence of Neurolymphomatosis	Chickens in Experiment	Incidence of Neurolymphomatosis	
Nov. 5-18.....	31	7	65	1	122	3*	218
Nov. 19-Dec. 9.....	42	2	62	2	133	1*	237
Dec. 17-30.....	48	3	62	0	112	1†	222
Dec. 31-Jan. 13....	44	5	52	0	99	0	195
Jan. 14-27.....	67	4	72	0	112	1*	251
Jan. 28-Feb. 10....	66	4	67	2	118	0	251
Feb. 11-24.....	71	3	63	0	103	0	237
Feb. 25-March 10..	106	5	62	1	104	1‡	272
March 11-24.....	101	7	59	0	111	0	271
March 25-April 7..	87	8	65	1	107	1†	259
April 7-21.....	72	11	53	0	90	0	224
Aver. no. chickens in animal room...	67	5.1 (7.6%)	62	0.6 (1.0%)	111	0.7 (0.6%)	240

* In chicken inoculated with strain 1.
† In chicken inoculated with strain 12.
‡ In chicken inoculated with strain 13.

of the number of animals in the laboratory and of the number of chickens with either clinical paralysis or thickening of nerves due to lymphomatous infiltrations. This record, computed for approximately biweekly intervals (table 14), gives a fair estimate of the incidence of paralysis in our laboratories.

Table 14 demonstrates that neurolymphomatosis is transmissible by inoculations with tissues from paralyzed chickens, but it also shows that paralysis occurs among chickens inoculated with tissues that were derived from seemingly unparalyzed birds.

The chickens in the first group in table 14 were inoculated with material derived from paralyzed chickens. Neurolymphomatosis strains 5 and 6 have been described. Strain 4 was a transmissible strain of lymphomatosis that originated in a chicken unsuccessfully inoculated with tissues of Hodgkin's disease of man. After four

successive subpassages this strain was dropped. Lymphomatosis of this strain was in most instances associated with extensive infiltration of peripheral nerves and occasionally with leukemic blood pictures. It produced infiltration in the inoculated breast muscle. Strain 14 originated in a chicken that was inoculated with sarcoma tissue occurring in an uninoculated control chicken. This strain, too, was discarded after three successive passages, mainly because the disease that developed among its passages was similar to that produced by strains 5 and 6.

The chickens in the second group in table 14 were inoculated with strain 2, known to produce lymphomatosis with microscopic infiltration in the nerves.

The third group comprises all other chickens kept in the animal room including those inoculated with strains of leukosis and sarcoma that do not produce paralysis and a few uninoculated chickens. Five of the eight instances of paralysis recorded in this group occurred in chickens inoculated with strain 1. Last fall a small "epidemic" of neurolymphomatosis occurred among the chickens inoculated with this strain for reasons that are unknown. Most of the cases occurred in a group of eight chickens that received fractions of plasma obtained by ammonium sulphate precipitation at various hydrogen ion concentrations. It is uncertain whether the four instances of lymphomatosis were spontaneous, or whether the plasma of the donor carried the agent of neurolymphomatosis. Since January this year no instance of paralysis has been seen in chickens inoculated with strain 1, and the severe test of intraneural inoculation, the results of which have already been described, indicates that this strain does not produce neurolymphomatosis.

COMMENT

Two varieties of transmissible lymphomatosis are described in this report. One (strain 2) is characterized by large lymphocytes, produces lymphomatous tumors after intramuscular transmission, infiltrates the blood-forming organs, and is almost invariably associated with lymphatic leukemia. It almost invariably produces severe anemia, often infiltrates nerves, but seldom produces clinical paralysis. It is very rare as a spontaneous disease and is readily transmissible by cell-free material. The second type of transmissible lymphomatosis (strains 5 and 6, neurolymphomatosis) produces mainly lymphomatosis of the nerves, often associated with lymphomatosis of the viscera, seldom with lymphatic leukemia and never with anemia. It is characterized by a predominance of small lymphocytes and is frequent as a spontaneous disease, but its transmissibility by cell-free material is thus far unproved.

Strain 2 can be propagated readily by cell grafts, and only under exceptional conditions can the free virus stimulate normal cells to neoplastic growth. An ultramicroscopic cause of lymphomatosis of strains 5 and 6 is still unproved. Nevertheless, analogy with related diseases of fowls suggests that neurolymphomatosis is also a neoplasm produced by a filtrable virus. The disease has the histologic characteristics of a neoplasm. It occurs almost exclusively in young adult chickens. It is a very common disease, its incidence being, in our experience, greater than that of all other neoplasms together. The

transmitting agent circulates in the blood of paralyzed chickens in high concentration throughout the entire course of illness even though the disease is not associated with leukemia. Ease of transmission by material containing live cells and difficulty of transmission in the absence of these cells are characteristic of many chicken sarcomas as well as of chicken leukosis.

Recent suggestions [21] that all types of lymphoid leukosis or all types of leukosis including lymphomas are caused by a single virus are contradicted by our experience. Strains 1, 2 and 5 have been propagated side by side during the past year and introduced by similar routes of inoculation into experimental birds of the same age, breed and source, yet each strain retained its distinguishing features as shown in table 15.

TABLE 15.—*Comparison of Observations on Strains 1, 2, 5 and 6*

Points of Comparison	Strain 1	Strain 2	Strains 5 and 6
Paralysis	None	None or slight	Conspicuous
Infiltration of nerves	None	Slight	Extensive
Blood changes			
(a) Red cell system	Erythroleukosis	Anemia	None
(b) White cell system	None or myeloblastic leukemia	Increase of large lymphocytes and occasionally of myelocytes	None or increase of small lymphocytes
Bone marrow	Hyperplastic	Hyperplastic	Usually normal
Site of intramuscular inoculation	No alteration detected	Infiltrated by large lymphocytes	No alteration detected
Inoculated nerve	Normal	Thickened	Thickened

The suggestion that all agents of leukosis may produce sarcoma is likewise erroneous. Among approximately three hundred chickens used in the study of neurolymphomatosis, only one instance of sarcoma of the type described by Rous was observed. This sarcoma proved to be readily transmissible (strain 15), but it did not produce neurolymphomatosis.

It is difficult to preserve strains of neoplasms by intravenous passages in animals that are likely to develop similar diseases spontaneously.

Subpassages of neurolymphomatosis are best made by intraneural inoculation. The early recognition of the disease by means of the characteristic local infiltration facilitates the maintenance of the strain. Strain 2 is best carried on by intramuscular inoculations. Diffuse infiltration or tumors formed by large lymphocytes in the inoculated muscle and the subsequent leukemia aid in the diagnosis of this strain. Strain 1 is best maintained by intravenous injection of blood into young chickens; thus far it is the only strain of leukosis the agent of which has been readily preserved in the dry state.

Many variations of transmissible lymphomatosis are known in mice, including one that often causes paralysis,[30a] but lymphomatosis of numerous nerves and ganglions with slight or with no alteration in the blood-forming tissues is a unique phenomenon observed only in chickens. Neurolymphomatosis is very common among chickens throughout the world, but there is utter ignorance of the circumstances under which the spontaneous disease originates. The causation and pathogenesis of the disease are still obscure. If it may be assumed that the disease is a neoplasm produced by a filtrable agent, this agent may be either neurotropic or lymphocytotropic or both. If the malignant lymphocytes that infiltrate the nerves arise in the lymphoid tissues, why do they not form lymphomatous infiltrations in these tissues? If the assumed virus becomes attached to the nerve and attracts lymphocytes, why did we fail to produce lymphoid infiltration in the nerves inoculated with cell-free virus? If the cells are malignant lymphocytes that contain no virus, what are the natural means that bring about the malignant transformation of lymphocytes, endowing them with special affinity for nerve tissues?

V. SUMMARY AND CONCLUSIONS

The virus of leukosis strain 2 brings about neoplastic growth of lymphocytes with characteristic morphologic and biologic properties. Injected intramuscularly it produces no tumors at the site of injection. The neoplastic cells produced by it in the blood-forming organs behave like the leukemic lymphocytes of mammals and can be grafted readily in muscle tissue, where they undergo autonomous multiplication and produce tumors.

Two transmissible strains of neurolymphomatosis (strains 5 and 6) that can be readily passed from diseased to healthy chickens by an inoculum containing viable lymphocytes are described. The transmitting agent became inactivated by freezing at —30 C. for thirty minutes or by drying from the frozen state.

The blood of paralyzed chickens contains the transmitting agent in high concentration during the entire course of illness, and when introduced by the intravenous route it produces neurolymphomatosis indistinguishable from the spontaneous disease. Blood cells readily transmit the disease, but plasma does not. The transmissibility of the disease by a virus, though probable, is not proved.

After intraneural injection of tissues from paralyzed birds the inoculated nerve becomes greatly thickened, and general neurolymphomatosis follows. The disease spreads along the course of the nerve fibers, but the cord is seldom infiltrated, and distant lesions are probably the result of hematogenous spread.

Lymphomatous infiltration and tumors of the viscera may be caused by the same virus that produces neurolymphomatosis.

Neurolymphomatosis is usually unassociated with morphologic blood changes, but in rare instances it is associated with lymphoid leukemia.

Neurolymphomatosis is a neoplastic disease allied to leukosis and sarcoma, but it is not produced by the agent that causes erythroleukosis and myeloid leukosis; neither does the agent of neurolymphomatosis produce erythroleukosis and myeloid leukosis.

Case Reports

GUMMA OF THE HEART

REPORT OF TWO CASES

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NEW YORK

Gumma of the heart undoubtedly existed unrecognized for centuries. Because of the disinclination of early nineteenth century physicians to perform necropsies in cases of inveterate syphilis,[1] it was not until 1845 that the first positive report of cardiac gumma was published, that by Ricord,[2] a syphilodermatologist.

Soon after this, Virchow[3] divided tertiary syphilis of the heart muscle into fibrous and gummatous types. The former is generally held to be indistinguishable from diffuse myofibrosis found with coronary sclerosis, although Takata[4] stated his belief that the syphilitic type can be recognized histologically. I am concerned here neither with the fibrous type of myocardial syphilis nor with the unconfirmed lesions described by Warthin[5] but with the gummatous type, which constitutes a distinct anatomic entity.

Gummatous myocarditis may be diffuse or localized and occurs in congenital as well as in acquired syphilis.

DIFFUSE GUMMATOUS MYOCARDITIS

Diffuse gummatous myocarditis unassociated with grossly recognizable gummas is extremely rare; only seven cases could be found recorded in the literature.[6] It is more often found in combination with

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1. Wilks, Samuel: Biographical Reminiscences, London, Adlard & Son, 1911, p. 17.

2. Ricord: Gaz. d. hôp., Aug. 30, 1845, no. 101, p. 402.

3. Virchow, R.: Virchows Arch. f. path. Anat. **15**:217, 1858.

4. Takata, F.: Virchows Arch. f. path. Anat. **228**:426, 1920.

5. Warthin, A. S.: Am. J. Syph. **2**:425, 1918.

6. (*a*) Adler, I.: New York M. J. **68**:577, 1898 (cases 5 and 6). (*b*) Thorel, C.: Virchows Arch. f. path. Anat. **158**:271, 1899. (*c*) Busse, O., and Hochheim, W.: Arch. f. Ophth. **55**:222, 1903. (*d*) van Huellen, A.: Ztschr. f. Heilk. **6**:227, 1905. (*e*) Berblinger, W.: Zentralbl. f. allg. Path. u. path. Anat. **21**:1045, 1910. (*f*) Salles, P. H. E.: Contribution à l'étude anatomo-pathologique de la pancardite syphilitique, Thèse de Paris, Paris, Ollier-Henry, 1918 (case 3).

the localized type (gumma). It is characterized by the presence of extensive interstitial and perivascular granulation tissue infiltrated by lymphocytes and plasma cells. Occasionally giant cells are present. There are also obliterative endarterial lesions and frequently miliary areas of coagulation necrosis. These necrotic foci are sometimes of submiliary size, but when larger constitute a stage of transition to the well localized gumma. The myocardial fibers may show atrophy and fatty change. There may be no macroscopic lesions or, at most, innumerable whitish flecks.

The diagnosis can be made only by microscopic examination. Because of the inconstant appearance of necrosis and giant cells and the frequent difficulty in differentiating less characteristic lesions from those of tuberculous etiology, Saltykow [7] and Baumgartner [8] placed the lesions of diffuse gummatous and tuberculous myocarditis in the same group and designated them as "specific productive myocarditis." Spirochetal stains are generally negative in tertiary syphilis and, therefore, often of no practical aid in diagnosis.

LOCALIZED GUMMATOUS MYOCARDITIS (CARDIAC GUMMA)

The localized type of gummatous myocarditis known as the gumma is decidedly more common and characteristic, possessing the same appearance and structure as gummas elsewhere in the body. A total of ninety-seven authentic cases of cardiac gumma have been reported.[9]

7. Saltykow, S.: Verhandl. d. deutsch. path. Gesellsch. **17**:321, 1914.

8. Baumgartner, H.: Frankfurt. Ztschr. f. Path. **18**:91, 1916.

9. (*a*) Dandridge, N. P.: M. & S. Reporter **28**:352, 1873. (*b*) Adler[6a] (case 7). (*c*) Luce, H.: Deutsches Arch. f. klin. Med. **74**:370, 1902. Fahr, T.: Virchows Arch. f. path. Anat. **188**:562, 1907. (*d*) Stockmann, W.: Ueber Gummiknoten im Herzfleische bei Erwachsenen, Wiesbaden, J. F. Bergmann, 1904 (collected fifty-six cases, including four of his own). (*e*) Handford, H.: Brit. M. J. **2**:1745, 1904. (*f*) Keith, A., and Miller, C.: Lancet **2**:1429, 1906. (*g*) Ashton, T. G.; Norris, G. W., and Lavenson, R. S.: Am. J. M. Sc. **133**:28, 1907. (*h*) Huchard, H., and Fiessinger, N.: Rev. de méd., Paris **27**:948, 1907. (*i*) Robinson, G. C.: Bull. Ayer Clin. Lab., Pennsylvania Hosp. **4**:1, 1907. (*j*) Vaquez and Esmein: Presse méd. **15**:57, 1907. (*k*) Heineke, A.; Müller, A., and von Hösslin, H.: Deutsches Arch. f. klin. Med. **93**:459, 1908. (*l*) Handwerch, C.: München. med. Wchnschr. **56**:916, 1909. (*m*) Klages: ibid. **59**:1323, 1912. (*n*) Letulle, M.: Bull. Soc. anat. de Paris **87**:31, 1912; ibid **89**:402, 1914-1919. (*o*) McWeeney, E. J.: Tr. Roy. Acad. Med., Ireland **31**:413, 1913. (*p*) Lombardo, G.: Pathologica **6**:83, 1914. (*q*) Soprana, F., and Piazza, C.: Riforma med. **31**:537, 567 and 590, 1915 (two cases). (*r*) Holterdorf, A.: München. med. Wchnschr. **63**:1651, 1916. (*s*) Reinhardt: ibid. **64**:1467, 1917. (*t*) Husche, K.: Ein Fall von Gummosis des Herzens, Inaug. Dissert., Berlin, 1918. (*u*) Salles[6f] (case 1). (*v*) Bridgman, E. W., and Schmeisser, H. C.: Johns Hopkins Hosp. Rep. **18**:90, 1919. (*w*) Takata[4] (four cases). (*x*) Girdwood, R. L.: M. J. South Africa **16**:183, 1920-1921. (*y*) Spalding, E. D., and Von Glahn, W. C.:

(Footnotes continued on next page)

The gumma may be solitary, but usually it is multiple and nodular, although serrated and jagged surfaces may be seen. It varies from pinhead size to that of a fowl's egg and is usually dull yellowish or grayish white, often with a bacon-like appearance. Palpation gives a firm, elastic, rubber-like sensation, from which the gumma derives its name (*Gummigeschwulst*). The older, more chronic lesion is generally surrounded by an irregular dense fibrous capsule which contributes to the firmness of the entire nodule.

The most frequent site of the cardiac gumma is the left ventricular myocardium, particularly the basal portion of the interventricular septum. Gummas in this location often give rise to heart block. Interference with cardiac function also arises when gummatous lesions compromise or invade the valves (table). A number of cases of involvement of the papillary muscles have been reported,[10] in one instance with

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Bull. Johns Hopkins Hosp. **32**:30, 1921. (*z*) Major, R. H.: Arch. Int. Med. **31**: 857, 1923. (*aa*) Friedman, W.: Proc. New York Path. Soc. **24**:24, 1924. (*bb*) Young, W. A.: Tr. Roy. Soc. Trop. Med. & Hyg. **19**:87, 1925-1926. (*cc*) Cabot, R. C.: Facts on the Heart, Philadelphia, W. B. Saunders Company, 1926, p. 375. (*dd*) de Marval, L., and Vivoli, D.: Rev. Soc. argent. de biol. **2**:425, 1926; Rev. Soc. de med. int. y soc. de tisiol. **2**:397, 1926. (*ee*) Cleland, J. B.: M. J. Australia **14**:540, 1927. (*ff*) Jansen, H.: Virchows Arch. f. path. Anat. **264**:730, 1927 (three cases). (*gg*) Cookson, H.: Brit. M. J. **2**:94, 1929. (*hh*) Hajoshi, I.: Ztschr. f. Kreislaufforsch. **21**:34, 1929. (*ii*) Staemmler: Verhandl. d. deutsch. path. Gesellsch. **25**:262, 1930. (*jj*) Kux, E.: Ztschr. f. Kreislaufforsch. **24**:1, 1932.

In addition, twenty cases of cardiac gumma have been found in which a description is absent or inadequate: Goodhart, cited by Hall, D. G.: Edinburgh M. J. **14**:322, 1903. Renvers, R.: Therap. d. Gegenw. **6**:433, 1904 (three cases). Schmorl: München. med. Wchnschr. **54**:285, 1907 (two cases). Brooks, H.: Am. J. M. Sc. **146**:513, 1913 (five cases). Welch, cited by Cabot.[9cc] Clawson, B. J., and Bell., E. T.: Arch. Path. **4**:922, 1927 (three cases). Coombs, C. F.: Lancet **2**:227, 281 and 333, 1930. Martland, H. S.: Am. Heart J. **6**:1, 1930-1931. Warthin, A. S.: ibid. **6**:163, 1930-1931 (two cases). Army M. Museum, Washington, D. C., Accession no. 26893, courtesy of Major V. H. Cornell, Medical Corps, U. S. A.

There have also been reported a number of instances of cardiac gumma which lack sufficient criteria for inclusion among the authentic cases: Israel, O.: Berl. klin. Wchnschr. **32**:792, 1895. von Genersich, A.: Pest. med.-chir. Presse **33**:84, 108, 1897. Jagic, N.: Ztschr. f. klin. Med. **66**:183, 1908. Rosenfeld, F.: Deutsches med. Wchnschr. **40**:1044, 1914. Macfie, J. W. S., and Ingram, I.: Ann. Trop. Med. **14**:147, 1920. Morin, H. G. S., and Fabre, H.: Marseille-méd. **60**: 1430, 1923.

10. Burney, Yeo, quoted by Phillips, S.: Lancet **1**:223, 1897. Cayley, W.: Tr. Path. Soc. London **26**:32, 1875. Jürgens: Berl. klin. Wchnschr. **28**:1031, 1891. Rolleston, H. D.: Tr. Path. Soc. London **44**:37, 1893. Lorrain: Bull. Soc. Anat. de Paris **70**:693, 1895. Stockmann[9d] (case 2). Ashton, Norris, and Lavenson.[9g] Spalding and Von Glahn.[9y]

rupture and sudden death.[9y] The endocardium or the epicardium overlying a subjacent gumma may show localized nonspecific sclerotic thickenings; infrequently, however, they may be involved by the specific gummatous process. In rare instances partial obliteration of the pericardial cavity may be produced.

The fate of a gumma in the heart is identical with that of gummas in other organs. Because of a marked tendency to fibrosis it may heal entirely, occasionally with calcification. More rarely it may undergo extensive softening with the formation of cavitations, in which event communication with the endocardial chambers is frequently established and cardiac aneurysm results.[11] A reported instance of embolization from such a source could not be confirmed.[12] Communication with the pericardial cavity is rarer and invariably associated with a complete rupture of the cardiac wall through the aneurysmal portion; this is manifested by sudden death.[13] Perforation of the interventricular septum by a gumma has been reported in congenital syphilis,[14] but the pathologic diagnosis is not conclusive.

The histologic diagnosis of cardiac gumma is based on the characteristic finding of a more or less round central necrotic area containing nuclear and cytoplasmic débris and occasionally remnants of myocardial fibers. The last point has been stressed in the literature as being of diagnostic aid in differentiating it from the tubercle. Surrounding this central area of coagulation necrosis there is a fairly well defined zone of granulation tissue, usually densely infiltrated by lymphocytes, plasma cells and fibroblasts. Giant cells, occasionally of the Langhans type, epithelioid cells and frequently eosinophilic leukocytes may be seen. Lesions of the vessels in this zone may be entirely absent. More frequently endarterial or endophlebitic hyperplastic processes, often with perivascular accumulations of round cells, are evident. In larger and more chronic lesions the entire inflammatory focus is usually, in turn, surrounded by a dense connective tissue capsule, from which fibrous strands radiate between the bundles of adjacent myocardial fibers. The gumma as a whole has a pronounced fibrous aspect.

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11. (*a*) McNalty, G. W.: M. Times & Gaz. **1**:624, 1873. (*b*) Goodhart, J. F., and Green, A. W.: Tr. Path. Soc. London **38**:102, 1887. (*c*) Pitt, G. N.: ibid. **42**:61, 1891. (*d*) Kockel, R.: Arb. a. d. med. Klin. zu Leipzig **1**:294, 1893. (*e*) Krönig: Berl. klin. Wchnschr. **32**:969, 1895. (*f*) Stolper, P.: Bibliot. med. (pt. C) **6**:25, 1896. (*g*) Jodlbauer, A.: Ein Fall von Syphilis des Herzens, Inaug. Dissert., Munich, 1897. (*h*) Duckworth, D.: Tr. Clin. Soc. London **29**:7, 1896. (*i*) Dandridge.[9a] (*j*) Luce.[9c] (*k*) Klages.[9m] (*l*) Young.[9bb] (*m*) Cookson.[9gg]

12. Oppolzer, quoted by Lang, T.: Die Syphilis des Herzens, Vienna, Wilhelm Braumüller, 1889.

13. Goodhart and Green.[11b] Pitt.[11c] Krönig.[11e] Dandridge.[9a]

14. Hughes, W. E.: Proc. Path. Soc., Philadelphia **3**:17, 1899-1900.

Cardiac gummas are rarely diagnosed clinically, although their presence can occasionally be suspected. At times, the lesion is an accidental postmortem finding which caused no symptoms during life. The following cases illustrate these points.

REPORT OF CASE 1

Gummas of the Left Ventricular Myocardium and Interventricular Septum; Necrosis, Cavitation and Communication with the Left Ventricular Cavity; Saccular Protuberance into the Pulmonary Subvalvular Region Producing Pseudostenosis.

A 53 year old Italian man was admitted to the service of Dr. B. S. Oppenheimer on Dec. 8, 1931, complaining of weakness, cough, anorexia, a pressing sensation in the left lower part of the chest radiating to the left upper part of the chest on walking, dyspnea on exertion and swelling of the ankles. The symptoms were of three months' duration.

There was slight cyanosis of the finger-tips and lips. The patient was mildly orthopneic. The temperature was 98.8 F., the pulse rate 116, and the respiratory rate 28 per minute. The pupils were regular and equal and reacted to light and in accommodation. There was dulness at the extreme base of the right lung with moist râles at the bases of both lungs. The heart was enlarged toward the axilla and extended about 5 cm. to the left of the sternum in the second intercostal space. The right border was percussed 4 cm. to the right of the sternum. At the base of the heart and just to the left of the sternum, there could be felt a strong systolic impulse fading in intensity toward the apex, where it was practically impalpable. A loud, sharp systolic first sound and a whistling systolic murmur were audible in the same area with the same distribution of intensity. At the apex there was a short systolic bruit transmitted into the upper part of the axilla and around into the left interscapular region. The second cardiac sound was barely audible. Occasional interpolated cardiac beats were present, the rhythm being otherwise regular. The pulses were equal, synchronous and of good quality. The liver was felt 4 fingerbreadths below the right costal margin; its edge was soft and round. Edema of the feet was present. Several serpiginous and round, paper-thin scars were observed over the right calf. The neurologic status was negative.

The Wassermann and Kahn reactions of the blood were both positive (4+). The systolic blood pressure was 120 mm. of mercury and the diastolic 65. The venous pressure was 9.5 cm. of blood (direct method). The hemoglobin was 100 per cent (17 Gm. per hundred cubic centimeters of blood). The leukocyte and differential counts were normal. The blood urea nitrogen was 42 mg. per hundred cubic centimeters. The urine contained a small amount of albumin.

A roentgenogram of the chest (fig. 1 *A*) showed a marked enlargement of the heart to the right and left. A prominence, roughly the size of a large plum, was evident in the region of the pulmonary artery.

An electrocardiogram revealed the rhythm to be very irregular owing to a combination of occasional sinus block with numerous nodal beats. The main deflection was very low, notched and widened (0.12 second) in all leads and partly inverted in lead III. The T-wave was low in all leads and inverted in leads I and II. The curve was thought to be suggestive of extensive myocardial damage with disturbance of the pacemaker. Three tracings showed essentially the same abnormalities.

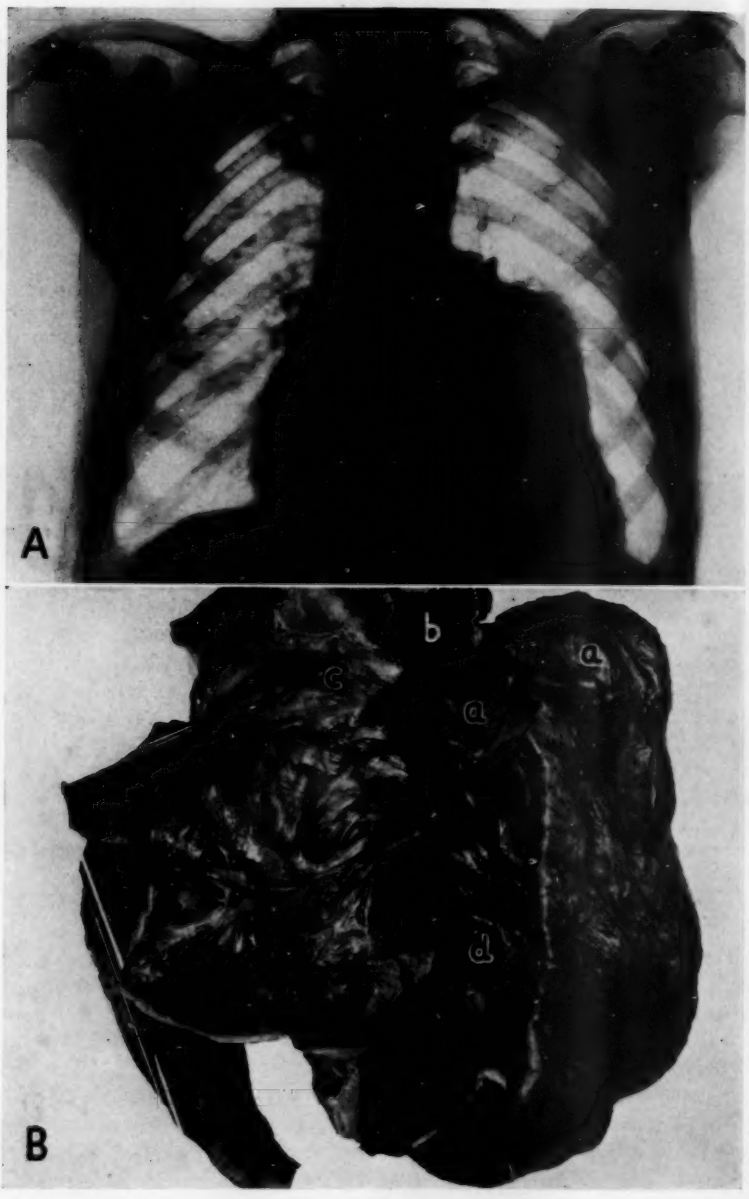

Fig. 1 (case 1).—*A*, roentgenogram of the chest, showing marked enlargement of the heart to the right and left. The large prominence in the region of the pulmonary conus was shown to be due to a gummatous aneurysm at the base of the left venticle. *B*, anterior view of the heart. Note the large saccular eminence (*a*) subjacent to the pulmonary conus (*b*) and protruding well into the pulmonary subvalvular region (*c*), creating the effect of a pseudostenosis. Near the apex on the septal wall is a firm yellowish myocardial gumma (*d*).

Despite diuretic and bismuth therapy, the patient grew steadily worse and died suddenly on the nineteenth day after admission.

The final clinical impressions included cardiac insufficiency, aortic stenosis, syphilis and a congenital cardiac lesion.

Autopsy.—The heart weighed 520 Gm. It was irregularly enlarged and somewhat distorted (figs. 1 *B* and 2). The visceral pericardium, particularly over the left ventricle and to a lesser extent over the right ventricle, had undergone a yellowish, somewhat nodular transformation. The most conspicuous distortion in the shape of the heart was due to the appearance of an irregularly rounded protuberance which appeared to be a continuation of the upper part of the anterior surface of the left ventricle. This protuberance was somewhat pear-shaped, and its apex extended approximately 3 cm. below the auriculoventricular sulcus anteri-

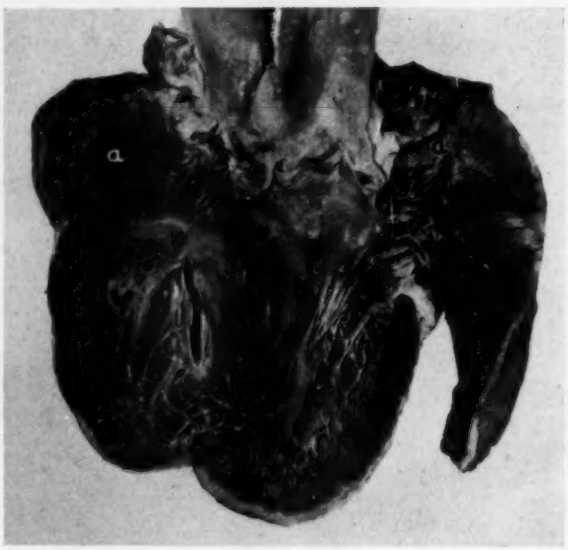

Fig. 2 (case 1).—View of the left side of the heart showing the large trabeculated communicating aneurysmal cavities (*a*) at the base of the left ventricle and anterior portion of the interventricular septum. The aorta grossly shows no lesions suggestive of syphilis, although there is definite microscopic evidence of such involvement at the root.

orly. Its upper portion reached approximately 4 cm. above the sulcus, abutting against the left lateral surface of the pulmonary conus. The left border of the pear-shaped mass extended over the margo obtusus and abutted against the left auricular appendix, which was raised by it to an upright perpendicular position.

The right ventricle was dilated. The columnae carneae were atrophic and flattened. The endocardium was somewhat thickened and whitened. Near the apex (on the septal surface) of the right ventricle was a shiny, yellowish, firm mass about the size of a hazelnut, shining through the endocardium. The outflow tract of the right ventricle was enlarged and presented a saccular aneurysm on its left wall. This aneurysm was the size of a pigeon's egg. It abutted against the left pulmonary cusp, extending 0.5 cm. above it. The aneurysmal mass was

covered by rough endocardium and formed a definite obstruction to the right outflow tract. The pulmonary cusps and artery were normal.

The left ventricle was atrophic; the outflow tract was enlarged and showed irregular whitened endocardial areas. One of these (on the septal surface) overlay a hyaline connective tissue mass which merged with the gumma described in the right ventricle. The papillary muscles were normal. Immediately anterior to the right aortic cusp and situated on the interventricular septum was a large foramen which opened into the irregular pear-shaped prominence seen on the outer surface. This prominence was seen to consist of a sacculated aneurysmal dilatation of the left ventricle, one of the saccules forming the aneurysmal bulging which obstructed the right outflow tract. The sac was, on the whole, smooth and lined with endocardium. Portions of it, however, presented a thin, furrowed thrombotic covering. The right portion of the left aortic cusp was impinged on and involved by the aneurysmal bulging. The remainder of the cusp was normal. The right and posterior aortic cusps were normal.

The aorta showed mild atheromatous changes but no lesions characteristic of syphilis. The orifice of the left coronary artery was somewhat narrowed; that of the right coronary artery, markedly narrowed. The major coronary arteries, the right more than the left, presented slight atheromatous lesions without narrowing. Both auricles and auriculoventricular valves were normal.

Microscopic Examination.—Aorta: The root of the aorta near the left-right commissure was the site of an advanced adventitial and periaortic inflammatory lesion. The walls of the vasa vasorum were extremely thickened, chiefly by intimal hyperplasia, which in many caused great narrowing of the lumen. Diffuse and perivascular infiltrations of small round cells were noteworthy. The annulus fibrosus and the tissue behind it were markedly fibrosed, thickened and similarly involved by the inflammatory process, having a position adjacent to the fibronecrotic wall of the aneurysmal cavity at the base of the left ventricle. The periaortic subepicardial fat tissue in the angle between the root of the aorta and the aneurysmal wall was likewise implicated in the infiltrative process (see Epicardium). The media of the aortic root was relatively intact. There was slight fibro-elastic proliferation of the intima.

Left Aortic Cusp: This leaflet at its junction with the wall of the aneurysm was the site of an interstitial valvulitis consisting of diffuse capillarization and infiltration with lymphocytes, large mononuclear cells and fibroblasts, which extended uninterruptedly from the base through the entire length of the leaflet. The structure was tremendously thickened, chiefly by the addition of an inflamed broad fibro-elastic band of tissue lying adjacent to the hyperplastic ventricular layer of elastica. The latter, as well as the original fibrous and spongy layers, was likewise involved in the inflammatory process, which was obviously an extension from that of the contiguous structures at its root.

Wall of Aneurysm at Base of Left Ventricle: On the internal surface was an adherent thrombus undergoing organization at its base. In the center of the wall was a large, more or less circular, homogeneous area undergoing coagulation necrosis. Surrounding it was a narrow zone densely infiltrated with lymphocytes, plasma cells, large mononuclear cells and fibroblasts. External to this was a dense fibrous capsule which, in one area, was the site of marked capillarization and formation of granulation tissue (fig. 3*A*). Giant cells were absent. The Levaditi stain was negative.

Another portion of the wall disclosed an older, more fibrotic lesion characterized by many small focal and confluent areas of coagulation necrosis situated in the interstices of an extensively sclerotic, infiltrated and capillarized band of tissue.

In the angle at the junction with the dome of the left ventricular myocardium, the fibrous aneurysmal wall diffused into the subepicardial fat tissue, giving the appearance of an extensive granulomatous lesion, replacing the fat cells and containing numerous thick-walled vessels with endarterial lesions, dense focal and diffuse accumulations of round cells and fibroblasts and excessive fibrous tissue proliferation. An occasional lymphatic channel was loaded with lymphocytes.

Epicardium: The subepithelial connective tissue layer of the epicardium was markedly thickened and fibrotic. The superficial zone of the subepicardial fat tissue (fig. 3*B*) was massively infiltrated by lymphocytes, plasma cells, large mononuclear cells and fibroblasts, with an occasional giant cell. This layer was vascularized by excessive numbers of young and old, thick-walled capillaries, with

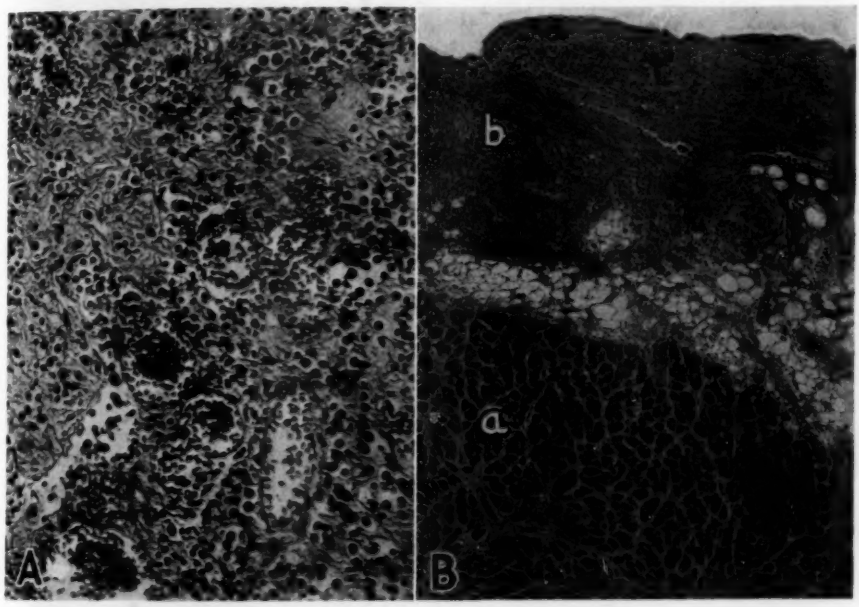

Fig. 3 (case 1).—*A*, granulation tissue surrounding a gumma in the wall of the aneurysm; dense infiltration by lymphocytes, plasma cells, wandering cells and fibroblasts with numerous capillaries. Hematoxylin and eosin; obj. 16 mm.; × 200. *B*, right ventricle (*a*) with overlying epicarditis (*b*). The granulomatous process consisting of dense round cell infiltration, fibroblasts, numerous capillaries and proliferated connective tissue is seen to occupy chiefly the superficial portion of the epicardium. The underlying myocardium in this section is relatively normal. Hematoxylin and eosin; obj. 25 mm.; × 50.

marked proliferation of the lining endothelial cells. The lymphatic channels were frequently packed with lymphocytes.

In general, the entire granulomatous lesion was situated at an appreciable distance from the underlying myocardium, although occasionally it dipped down to involve its surface. The myocardium of the right ventricle was extensively scarred. These large areas of fibrosis contained isolated muscle fibers, thick-walled vessels with intimal hyperplasia and narrow lumens and a scattering of lymphocytes.

Such a superficial scarred area was seen to send narrow fibrous strands into the epicardial lesion where they were in proximity.

Right Ventricle: A section through the firm yellowish lesion at the base of the trabeculum septomarginalis disclosed an area of coagulation necrosis identical with that noted in the wall of the aneurysm. The appearance was typical of gumma.

Other Organs: Significant pathologic observations in the other organs included chronic passive congestion of the liver, spleen and kidneys, a pulmonary infarct and fibrosis of the pancreas. Anatomic evidence of syphilis could not be demonstrated in any of the organs other than the heart.[15]

COMMENT ON CASE 1

The discovery of gummatous cardiac aneurysms in this patient was a complete surprise but explained the phenomena which were so puzzling during life, i. e., the signs of valvular stenosis, a prominence in the region of the pulmonary artery (by roentgenogram) and serologic findings indicative of syphilis.

The necropsy disclosed a rare consequence of cardiac gumma, i. e., caseation, cavitation and communication with the ventricular cavity. Thirteen instances of gummatous cardiac aneurysm have been observed.[11] (A number of so-called syphilitic cardiac aneurysms due to "syphilitic coronary endarteritis" have been excluded.) Lack of histologic detail and absence of investigation of the coronary system detract from the value of some of the earlier reports. Gummatous aneurysm, like the gumma, may be situated in any portion of the heart but almost always occurs in the left ventricle. In contrast to the usual apical position of cardiac aneurysm in consequence of coronary occlusion, the gummatous aneurysm, in the majority of cases, is found at the base of the ventricle behind either flap of the mitral valve or in the upper portion of the interventricular septum.

In this case the large saccular protuberance at the base of the left ventricle in the region of the margo obtusus (fig. 1 *B*) accounts for the unusual roentgen shadow in that situation (fig. 1 *A*). This contributed to the difficulty in arriving at a clinical diagnosis. The unusual cardiac physical signs together with the roentgen shadow presented a complex, rendered more enigmatic by the fact that the patient was syphilitic. It was difficult to correlate the latter finding with the observation of the maximum physical signs (strong systolic impulse, sharp first cardiac sound, and whistling systolic murmur) at the pulmonic area. A suspicion of a congenital cardiac lesion, such as pulmonic stenosis, was further strengthened by the absence of the second pulmonic sound; however, this was not compatible with the roentgen shadow in the region of the pulmonary artery. Nevertheless it was felt that the auscultatory cardiac signs were an expression of orificial stenosis, the exact situation of which was uncertain.

15. The brain was not examined.

Examination of the heart post mortem revealed this impression to be correct, although the mechanism was entirely unsuspected. Figure 1 *B* shows to what extent the gummatous aneurysm in the upper portion of the interventricular septum protruded into the region of the pulmonary valve to cause a pseudostenosis with consequent dilatation of the right side of the heart.

A number of cases have been recorded in the literature (table) in which obstruction in the outflow tract of the right ventricle was caused by a gumma protruding from the interventricular septum, but no other case could be found in which the syndrome of Bernheim was simulated by a gummatous aneurysm. Other instances of valvular or subvalvular stenosis or insufficiency have been reported in which the valve or juxtavalvular region was directly invaded or compromised by a gummatous process in the contiguous myocardium or great vessels (table). Gummas in the upper portion of the interventricular septum protruding into each ventricular chamber and causing both aortic and pulmonic stenosis were found in a case reported by Major.[9z] Similar biostial involvement was present in the cases of Luce [9c] (later reported by Fahr) and Robinson.[9l] Because of the relative frequency of gummatous changes in the upper portion of the interventricular septum and root of the aorta, it is not surprising that interference with valvular function occurs most frequently at the aortic and pulmonic valve regions. From the tabulation it may been seen that the order of descending frequency of such interference is pulmonary, aortic, tricuspid and mitral. Acquired syphilis has not yet been proved to originate in a valve. Staemmler [9ll] pointed out that the gummatous process in the mitral valve in his case originated from the root of the aorta.

Of further pathologic interest in this case was the marked chronic epicarditis (fig. 3 *B*), which was most evident at the base of the ventricles. This inflammatory granulomatous lesion was undoubtedly syphilitic and was seen to originate by extension from the gummatous wall of the aneurysms. A similar lesion, but lacking vascular changes, was noted by Thorel [6b] in a case of diffuse gummatous myocarditis, and was apparently the first of the type to be reported. Salles [6f] published excellent descriptions and photomicrographs of the characteristic lesion, which in his case arose from sclerogummatous nodules in the interaortopulmonary subepicardial region. "Syphilitic epicarditis" with miliary gummas was likewise described by Letulle.[9n] Identical microscopic alterations of the epicardium were found in a case of congenital cardiac gumma by Oberhammer.[16]

16. Oberhammer, K.: Ztschr. f. Kreislaufforsch. **19**:9, 1927.

Macroscopically, the epicardium may be granular as well as thickened.[17] Girdwood[9x] observed a diffuse thickening of the epicardium extending upward into the adventitial tissue around the intrapericardial portion of the aorta and pulmonary artery. There were no gross lesions of syphilitic aortitis in his case, although they were present in the cases of Salles and Letulle. Lesions histologically similar to those observed in this case are seen not infrequently in the epicardium and adventitia at the root of the aorta in cases of syphilitic aortitis. These lesions, too, undoubtedly represent extension from the inflamed aorta and occasionally are seen to extend a short distance down over the contiguous ventricular myocardium.

It is apparent that in cases of cardiac gumma, chronic granulomatous epicarditis is of syphilitic origin even though foci of coagulation necrosis and giant cells are absent. The visceral layer of the pericardium is involved by extension from contiguous myocardial (or aortic) gummas.

REPORT OF CASE 2

Gummatous Aortitis with Commissural Involvement; Gummas of the Myocardium; Rheumatic Mitral and Aortic Stenosis and Insufficiency.

The records in this case have been made available through the kindness of Drs. Antopol and Weiss of the Bayonne Hospital and Dispensary, Bayonne, N. J. A Negro, aged 40, entered the hospital moribund on Sept. 1, 1932, complaining of dyspnea, orthopnea, palpitations and swelling of the feet and abdomen for five months. He had had polyarthritis fifteen years before admission. Three years later he contracted a purulent urethral discharge and a sore on the penis. Two years prior to admission he was given a series of intragluteal injections, presumably for syphilis.

On examination he was dyspneic and orthopneic. Râles were heard throughout both lungs. A diffuse pulsation was present over the left lower part of the chest anteriorly. The heart was enlarged to the left; its apex was in the sixth left intercostal space at the anterior axillary line; the right border of the heart was percussed 1 fingerbreadth to the right of the sternum; there were systolic and diastolic murmurs over the entire precordium. The cardiac rhythm was completely irregular. The liver was enlarged, and there were signs of free fluid in the abdomen. Marked albuminuria was found. The patient died two days after admission.

Autopsy.—Only the heart and aorta are described in detail. The heart weighed 600 Gm. and was enormously enlarged. The right auricle was dilated. The tricuspid valve showed fusion of the septal-anterior commissure. The chordae tendineae to the anterior cusp were slightly thickened. The right ventricle showed marked dilatation of the inflow and outflow tracts. The pulmonary cusps were normal. The pulmonary artery at the base of the right-left commissure was slightly thickened. The pulmonary artery was somewhat dilated and delicately puckered and showed several small yellowsh plaques.

The left auricle was markedly dilated. The endocardium was thickened. The mitral valve showed marked universal thickening with stenosis of the orifice and fusion and thickening of the chordae tendineae. When the mitral ring was pal-

17. Wagner, K. E., and Qwiatkowski, G. I.: Virchows Arch. f. path. Anat. **171**:369, 1903. de Marval and Vivoli.[9dd]

pated, the posterior commissure presented a rubbery resistance to pressure. Sections through this region showed extensive scarring. In its center was a glistening, pale yellow, firm mass measuring 2 mm. in diameter and reaching almost to the endocardial surface under the mitral pocket. The entire posterior mitral pocket was covered with a thrombus. Another typical gumma was disclosed 1 cm. to the left of the one just described. The left ventricle was dilated and hypertrophied. The aortic cusps were thickened. The right-posterior and left-right commissures showed typical rheumatic fusion, but the left-posterior showed a typical syphilitic separation.

The aorta, starting with the insertion of the annulus and continuing up through the entire arch, showed porcelain-blue longitudinal wrinkling and puckering of the intima. The orifices of the coronary arteries were narrowed by the inflammatory process, the right more than the left. The coronary arteries were otherwise normal.

Microscopic Examination.—Aorta: The architecture was markedly distorted by an infiltrative and sclerotic process. The adventitia was tremenously thickened and fibrosed. There were marked obliterative endarterial proliferation of the vasa vasorum and dense diffuse and focal perivascular and perineural aggregations of lymphocytes and plasma cells. Large, irregular, dense fibrous scars, usually perivascular, were present in the media, with thickening, rupture and distortion of the elastic fibers. Innumerable capillaries with perivascular lymphocytic and plasma cell infiltrations were likewise found diffusely in the media. A few focal areas of necrosis with nuclear débris were situated in the outer third of the media. There was a noninflammatory dense fibro-elastic proliferation of the intima, which was most marked over the more advanced medial lesions.

Commissure Between Left and Posterior Aortic Cusps: The aortitic lesion just described continued unchanged into the region of the attachment of the two aortic cusps, which grossly showed separation. In addition, in the annulus fibrosus, there were giant cells in moderate number, occasionally resembling the Langhans type.

Left Aortic Cusp: The attached segment of aorta showed a lesion the same as that just described, descending into the wall of the sinus of Valsalva, but ending abruptly at the upper portion of the aortic annulus. The "ring spongiosa" [17a] of the aortic cusp contained several smooth muscle bundles and many capillaries and arteries. The vessels ascended in liberal numbers throughout the length of the valve cusp in the broad fibro-elastic reduplication situated on the ventricular side of the hyperplastic elastica ventricularis. Toward the closure line a moderate infiltration with lymphocytes was present in the reduplicated layer.

Posterior Leaflet of the Mitral Valve: The ring of the valve was fibrotic, vascularized and infiltrated with plasma cells and lymphocytes. The elastica auricularis at this point became remarkably hyperplastic, widened and deviated toward the ventricular side of the leaflet by broad hyperplastic fibro-elastic reduplications, themselves the site of marked vascularization and lymphocytic infiltration continuous with that at the ring.

Mitral Valve at Posterior Commissure: A section through the yellow nodule in the valve pocket showed a typical blood platelet thrombus in the pocket undergoing basal organization. It was thickest where it overlay the superficially situated necrotic nodule. The latter had a fairly homogeneous appearance in the center, where it was comprised of cytoplasmic and nuclear débris. This mass of necrotic tissue was surrounded by a more or less irregular zone of granulation tissue con-

17a. Gross, L., and Kugel, M. A.: Am. J. Path. **7**:445, 1931.

taining many capillaries and dense infiltrations of lymphocytes, plasma cells, fibroblasts and histiocytes. Delicate fibrous strands emerged from the inner aspect of this zone to invade the central necrotic area.

External to the active granulating zone was a dense, jagged fibrous capsule with prolongations along the adjacent subendocardium and between the myocardial fibers. These strands were likewise infiltrated with lymphocytes and, to a lesser extent, with plasma cells. They contained vessels in abundance, which in the region of the auriculoventricular junction showed extreme intimal hyperplasia, often with complete obliteration of the lumens. There were no giant cells.

Left Ventricle: There was a definite excess of perivascular fibrous tissue, as well as a slight, more diffuse interstitial fibrosis. No definite Aschoff bodies were found. There was moderate hyperplasia of the intima of the smaller and larger arteries, often localized in form. The epicardium was not thickened, but contained numerous foci of plasma cells and lymphocytes.

Pulmonary Artery and Valve: The pulmonary artery was normal. The ring spongiosa contained a few blood vessels and no cells. There were no inflammatory changes in the valve.

Commissure Between Right and Left Pulmonary Valve: The wedge of media at the root of the pulmonary artery which descends into the commissure was invaded by numerous blood vessels, which were surrounded in the outer zone by plasma cells. The adventitia and media were scarred and showed rupture and proliferation of the elastica. The valvular endocardium and intima in the commissural region were thickened by dense, vascularized scar tissue.

COMMENT ON CASE 2

This case represents the type in which cardiac gumma is an incidental finding at necropsy and has been unproductive of clinical manifestations. The site of the gumma was unusually firm to the touch. It was situated at the posterior commissure of the mitral valve, which was the site of rheumatic, sclerotic disease. On section, the firmness was found to be due largely to the dense, thick, fibrous capsule which enveloped the gumma.

The history of this patient included both polyarthritis and a penile lesion. Either rheumatic or syphilitic aortic insufficiency could have accounted for the clinical picture of cardiac decompensation. The necropsy disclosed the fact that both these lesions were present.[18]

RECAPITULATION

Acquired tertiary syphilitic disease of the heart (exclusive of syphilitic aortitis with commissural involvement) is infrequent. If one omits from consideration the controversial diffuse fibrous type, the disease may be said to assume the form of diffuse interstitial gummatous myocarditis or that of localized gummatous myocarditis. The latter type (cardiac gumma) is by far the more common lesion. Direct exten-

18. The association of rheumatic valvulitis with (gummatous) commissural syphilis in the same valve is a very rare postmortem observation (Sager, R. V., and Sohval, A. R.: Arch. Path. **17**:729, 1934).

Cases in Which Cardiac Valves Were Compromised Either by Direct Gummatous (or Granulomatous) Invasion or by Juxtaposed Gumma

Author	Valve Invaded	Origin of Syphilitic Process	Valve Compromised by Juxtaposed Gumma	Origin of Gumma
Wagner: Arch. d. Heilk. **7**: 518, 1866	.....	.....	Pulmonary	Interventricular septum
Schwalbe: Virchows Arch. f. path. Anat. **119**: 271, 1890	Pulmonary	Pulmonary artery	Pulmonary	Interventricular septum
Volmer: Ueber Gummata des Herzens, Inaug. Dissert., Kiel, 1893 (case 2)	.....	.....	Tricuspid	Right ventricle
de Massary: Bull. Soc. anat. de Paris **9**: 504, 1895	.....	.....	Aortic	Left ventricle
Luce (Fahr)[90c]	.....	.....	Pulmonary and aortic	Interventricular septum
Stockmann[90d]				
Case I-A	Pulmonary	Interventricular septum	Aortic	Interventricular septum
Case III	.....	.....	Pulmonary	Interventricular septum
Robinson[90i]	Aortic and tricuspid	Interventricular septum	Aortic	Interventricular septum
Klages[90m]	Aortic	Aneurysm of root of aorta, interventricular septum	.....	.....
Holterdorf[90r]	Pulmonary	Pulmonary artery	.....	.....
Bridgman and Schmeisser[90v]	Tricuspid	Interventricular septum	.....	.....
Girdwood[90x]	.....	.....	Pulmonary and tricuspid	Interventricular septum, right auriculoventricular septum
Spalding and Von Glahn[90y]	Aortic (loss of substance)	Aorta	.....	.....
Major[90z]	Aortic and pulmonary	Interventricular septum	Pulmonary	Interventricular septum
Friedman[90aa]	Mitral	Aorta (?)	.....	.....
Cleland[90ee]	.....	.....	Pulmonary	Interventricular septum
Gallavardin and Josserand: Lyon méd. **139**: 135, 1927	Aortic	Aorta (?)	.....	.....
Jansen (case 2)[90ff]	Aortic	Aneurysm at root of aorta	.....	.....
Staemmler[90ii]	Mitral	Aorta	.....	.....
Kux[90jj]	Pulmonary and tricuspid	Pulmonary artery, interventricular septum	.....	.....
Norris: U. S. Nav. M. Bull. **30**: 37, 1932	Aortic (loss of commissure)	Aneurysm in sinus of Valsalva	.....	.....
Case 1	Aortic	Interventricular septum	Pulmonary	Interventricular septum

Summary

	Aortic	Pulmonary	Mitral	Tricuspid
Cases with actual invasion of the valves	8	5	2	3
Cases with juxtavalvular gummas, interfering with function of valves	4	8	0	2
Total cases with interference in valvular function	12	13	2	5

sion to the valvular or mural endocardium and pericardium may occur. Rarely, the heart valves (table), auricles,[9t] epicardium or interventricular septum may be involved by direct extension from the roots of the aorta and pulmonary artery.

The two cases herein reported are examples of cardiac gummas differing markedly in the extent of damage produced. Case 1 is an instance of extensive gummatous aneurysm formation interfering with valvular function. On the other hand, case 2 was typical of the asymptomatic myocardial gumma. In neither case was the diagnosis made clinically. However, when atypical cardiac findings are observed in a patient with a condition known or suspected to be syphilis, tertiary cardiac syphilis (gumma) should be considered. The suspicion would be further enhanced in the presence of heart block, unusually situated weird stenotic murmurs and unexplained roentgen shadows at the borders of the heart. Positive serologic data may be lacking and are not essential for the diagnosis.

SUMMARY

Acquired tertiary syphilitic heart disease (exclusive of aortitis with commissural involvement) is uncommon and consists of circumscribed gummatous myocarditis (cardiac gumma) and diffuse gummatous myocarditis.

The authentic cases of cardiac gumma recorded in the literature have been enumerated; to these, two new cases are added.

Gummatous cardiac aneurysm generally occurs at the base of the left ventricle, where it is particularly apt to interfere with valvular function.

Involvement of the pericardium (especially of the visceral layer) occurs generally by extension from myocardial gummas and less often from gummatous lesions in the roots of the great arterial trunks.

Clinical recognition of the disease is rare. Unusually situated weird stenotic murmurs, unexplained roentgen shadows at the cardiac margins and heart block in a patient in whom syphilis is suspected suggest the possibility of tertiary cardiac syphilis, most likely gumma.

Laboratory Methods and Technical Notes

EMBEDDING IN GLYCOL STEARATE

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Orton and Post [1] in a search for an embedding material which would not interfere with the demonstration of fat in brain tissue tried di-glycol stearate. Having further tested this material as a general embedding agent, I have found a number of comparisons that may be made between it and paraffin, which it resembles in many respects.

Glycol stearate is a slightly yellowish, firm, waxy substance with a melting point near 50 C. It is water-diffusible. Tissue taken directly from an aqueous fixative and placed in the melted wax will become slowly impregnated by it. When melted, the wax is miscible in any proportion with 95 per cent alcohol. It is soluble in such substances as ether, chloroform, and xylene.

Blocks of tissue to be embedded are passed through graded percentages of alcohol as desired. They are then put into a mixture of equal parts of 95 per cent alcohol and glycol stearate and placed in an incubator at 56 C. for a period of from twelve to twenty-four hours. The tissues are then immersed in the pure melted wax and allowed to remain in the incubator for a period of time depending on the size of the blocks. If several blocks are being treated together, it is well to transfer them into fresh melted wax at least once during the process of impregnation. Blocks that are 2 mm. in thickness are thoroughly saturated by the wax at the end of twenty-four hours. If blocks are 4 mm. thick they should be left in the wax for forty-eight hours. Embedding is then performed in the same manner as when paraffin is used.

The time required for the process is greater than for some methods of embedding in paraffin, but the treatment with a mixture of alcohol and glycol stearate before the tissue is placed in pure wax materially hastens the process. In experimenting to find the proper length of time to leave the tissues in the melted wax, I have noticed that satisfactory sections can often be cut from blocks impregnated for only half the suggested time before embedding. When such blocks are examined a number of days or weeks after they have been embedded it is found that the tissues have shrunken away from the wax. This is obviously due to evaporation of the solvent remaining in the tissue and incomplete saturation of the tissue by glycol stearate.

When the blocks are removed from the molds after the wax has cooled and hardened, a little more care is required to avoid mutilation than when paraffin is used, for the stearate is more brittle than paraffin and adheres somewhat more persistently to the walls of the molds.

From the Pathology Department of the College of Medical Evangelists.

1. Orton, S. T., and Post, J.: Bull. Neurol. Inst. New York **2**:302 (July) 1932.

Sections are cut in the same manner as from paraffin blocks and nice ribbon sections can usually be made. As in the case of paraffin the sections sometimes roll up when cut. This is more liable to occur when the humidity or the temperature of the air in the room is low or when very thick sections are cut. As the melting point of glycol stearate is sharper than that of ordinary paraffin, the wax softens relatively little until it is heated to near this point. Therefore, in warm weather it is easier to cut sections from this material than from common paraffin.

Sections may be floated on the surface of warm water and then placed on slides or cover slips with a film of albumin fixative. The optimum temperature of the water to flatten the sections properly without melting the embedding material is 45 C.

The glycol stearate may be removed from the mounted sections by immersion for five minutes in chloroform or xylene, as in the case of paraffin-treated tissues. If all the wax does not appear to be dissolved at the end of five minutes, the quantity which remains usually will not interfere with staining. The alcohol baths commonly employed will aid in completing the removal of the glycol stearate.

Sometimes the sections fail to remain adherent to the slides when albumin fixative is employed. An alternative method of attaching sections to slides may be used with the aid of dropping bottles. Sections may be floated onto slides and then dried. The slides may be flooded with xylene for from two to five minutes, then blotted off and 95 per cent alcohol applied for one minute and blotted off. Thin pyroxylin may then be applied over the tissues as in the method frequently employed with frozen sections. Freshly embedded tissues always cut and mount better than those from older blocks.

The quality of the stained sections prepared by this method compares favorably with those embedded in paraffin. Cells show little distortion unless the blocks of tissue were not thoroughly impregnated and were allowed to dry out and shrink before being cut. Fats are too completely removed to be easily demonstrated in sections embedded by this method. The cost of the process is similar to that of embedding in paraffin.

General Review

WEIL'S DISEASE

REPORT OF A CASE WITH POSTMORTEM OBSERVATIONS AND REVIEW OF RECENT LITERATURE

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In 1886 Weil [1] described a disease in which sudden onset, prostration, fever, muscular pain, jaundice, hemorrhagic tendencies and renal injury were the characteristic features. Inada [2] in 1916 reported that a spirochete was the causative agent. Noguchi [3] classified this organism and named it Leptospira icterohaemorrhagiae. The disease is also called spirochetal jaundice or spirochaetosis icterohaemorrhagica.

Although many cases have been reported from Europe, Japan and the East Indies, this condition has apparently been extremely rare in the United States. Wadsworth [4] in 1922 observed the first proved case in this country and called attention to the fact that the disease could be acquired from the accidental prick of a needle containing a virulent culture obtained from a common rat which harbored the spirochete. Ten other cases have been reported since that time from New York,[5] Virginia,[6] District of Columbia,[7] Pennsylvania [8] and California.[9] No case has previously been recorded from New England.

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1. Weil, H. A.: Deutsches Arch. f. klin. Med. **39**:209, 1886.
2. Inada, R.; Ido, Y.; Hoki, R.; Kaneko, R., and Ito, H.: J. Exper. Med. **23**:377, 1916.
3. Noguchi, H.: J. Exper. Med. **27**:575, 1918.
4. Wadsworth, A.; Langworthy, V.; Stewart, C.; Moore, A., and Coleman, M.: J. A. M. A. **78**:1120, 1922.
5. (*a*) McDowell, E. S.: New York State J. Med. **25**:19, 1925. (*b*) Cushing, E. H.: J. A. M. A. **89**:1014, 1927.
6. Mulholland, A. B., and Bray, W. E.: J. A. M. A. **90**:1113, 1928.
7. Towler, H. H., and Walker, J. E.: J. A. M. A. **89**:86, 1927.
8. (*a*) Sailer, J.: Am. J. M. Sc. **170**:332, 1925. (*b*) Hayman, J. M., and Lynch, F. B.: ibid. **173**:8, 1927.
9. Ball, A. H.: Am. J. Clin. Path. **3**:283, 1933.

It is our purpose in this paper to add the twelfth proved case, to review the pathologic and clinical aspects of the disease, to discuss all the reported American cases and to point out certain facts derived from a study of the recent literature which will aid in the understanding of this disease entity.

The paucity of cases recorded in the United States may be due either to the rarity of the disease or to the fact that the clinical picture and the proper laboratory methods of diagnosis are not generally known. That the latter explanation seems more reasonable was well brought out by the extensive work of Schüffner [10] in the Netherlands. By means of a perfected agglutination test he was able to diagnose many cases not recognized clinically. Fairley,[11] having found 1 typical case in a sewer worker in London, applied the same agglutination test to all sewer workers who gave a past history of any illness resembling Weil's disease, and he discovered 7 instances in which the disease had not originally been diagnosed. Davidson,[12] in Scotland, using a similar method, was able to collect 23 cases among fish cutters, whose trade had not previously been suspected of exposing one to the organism. These and similar studies have resulted in a sharp increase in the number of cases reported from England, the Netherlands and other countries.

It has been known since the World War [13] that jaundice is not constantly present in Weil's disease. In the literature since that time this point has either not been mentioned or considered as a clinical oddity. However, a study of cases of the disease in the Netherlands, England and France in recent years in which Schüffner's diagnostic procedures were used revealed the significant fact that over 50 per cent of patients with Weil's disease do not have jaundice. In consequence, it has become the practice of European workers to refer to the condition as Weil's disease in preference to those synonyms which emphasize "jaundice." A similar nomenclature should be adopted in this country.

REPORT OF CASE

History and Course.—The patient, a 38 year old white man (a fish cutter), entered the Fifth Medical Service of the Boston City Hospital at 5 p. m. on May 31, 1934, with the complaint of severe prostration and jaundice. The history was secured from his family, as the patient was too ill to answer rationally.

The patient was in excellent health until seven days before admission to the hospital, when he noticed lassitude, anorexia and progressive pains and weakness in the muscles of both legs. At about the same time rigor of moderate severity developed followed by high fever. By the end of the first day he was forced to

10. Schüffner, W.: Tr. Roy. Soc. Trop. Med. & Hyg. **28**:7, 1934.

11. Fairley, N. H.: Brit. M. J. **2**:10, 1934.

12. Davidson, L. S.; Campbell, M. A.; Rae, H. J., and Smith, J.: Brit. M. J. **2**:3859, 1934.

13. (*a*) Valassopoulo, A.: Bull. et mém. Soc. méd. d. hôp. de Paris **41**:920, 1917. (*b*) Dawson, B.; Hume, W., and Bedson, S.: Brit. M. J. **2**:345, 1917. (*c*) Stokes, A.; Ryle, J. A., and Tytler, W. H.: Lancet **1**:142, 1917.

remain in bed. He continued in this condition for four days, when it was noticed that his skin was "yellow" and the urine dark red. No attention was given to the character of the stools. After the onset of the jaundice there were nausea and vomiting but no abdominal pain. Severe hiccups developed and persisted for three days. The family noticed that during all this time he appeared unusually weak and was unable to attend to even minor personal duties. The patient continued to fail rapidly and was sent to the hospital on the eighth day of his illness.

The family and the social history, except the patient's trade, were of no significance.

The family stated that the patient had never been sick since childhood and had not missed a day of work for over a year. He had never received antisyphilitic therapy or other medication. There was no history of the use of alcohol.

On admission the temperature was 99 F., the pulse rate 100 per minute and the respiratory rate 34. Examination revealed a markedly jaundiced man with dull senses, who was severely prostrated and able to utter only a few unintelligent words. He hiccuped persistently. There was no stiffness of the neck. The sclerae and skin were intensely icteric and the conjunctivae moderately injected. The pupils were small and regular in shape and reacted sluggishly to light. A slight herpetic eruption was present on the lips. The tongue was dry and cracked, and there was much dried blood on the dorsum and about the lips. The gingival margins were soft and bled on slight trauma. No injection of the blood vessels of the pharynx was noticed. There was no evident adenopathy. Auscultation of the chest revealed nothing abnormal except a few scattered moist râles. The heart sounds were rapid and of poor quality, but of regular rhythm. On percussion the borders of the heart seemed within normal limits. The blood pressure was 120 mm. of mercury systolic and 50 mm. diastolic. The pulse was regular but of poor volume. The abdomen was distended. On percussion the liver seemed enlarged, but owing to the distention it could not be satisfactorily palpated. The spleen was not palpable. The genitalia were normal. Swelling and tenderness were marked in the muscles of the right thigh and less evident in other groups of muscles. The tendon reflexes were present but sluggish. No pathologic reflexes were elicited. An examination of the rectum gave negative results.

The urine was concentrated and bile-stained and contained albumin and granular casts. The white blood cell count was 28,750 per cubic millimeter, while the red cell count was 3,130,000. The hemoglobin reading was 68 per cent on the Sahli scale. A stained blood smear revealed a differential count of 95 per cent polymorphonuclear leukocytes and 5 per cent lymphocytes.

The report of the chemical analysis of the blood was as follows: nonprotein nitrogen, 200 mg. per hundred cubic centimeters; sugar, 55 mg.; total protein, 4.4 Gm., with an albumin-globulin ratio of 31 : 69; cholesterol, 90 mg.; calcium, 10 mg., and phosphorus, 13.6 mg. One culture of the blood resulted in a growth of Staphylococcus aureus. The stool was liquid and pale tan and gave a strongly positive reaction to the benzidine test for occult blood. The Wassermann reaction was negative on two occasions.

The patient lapsed into coma shortly after admission. He received 1,500 cc. of a physiologic solution of sodium chloride by hypodermoclysis, 100 cc. of a 50 per cent solution of dextrose and an ampule of calcium gluconate intravenously, caffeine and an enema.

The respirations became of the Cheyne-Stokes type and the pulse thready. The chest began to show many scattered moist râles, and the pupils were dilated. The temperature rose to 100.5 F. Death occurred at 9 a. m. on June 1, 1934, after sixteen hours of hospitalization. An autopsy was performed.

Macroscopic Examination.—The body was that of a well developed, well nourished white man with generalized jaundice (the skin was "bright orange-yellow").

Peritoneal Cavity: About 20 cc. of clear orange-brown fluid was present.

Pleural Cavity: A few fibrous adhesions bound the upper lobe of the left lung to the anterolateral portion of the parietal pleura.

Heart: The heart weighed 340 Gm. and appeared normal.

Lungs: The lungs weighed 750 and 730 Gm., respectively, and were congested and edematous, yielding a thin, pale red fluid on pressure. The trachea and bronchi were normal.

Spleen: The spleen weighed 120 Gm. and was somewhat soft.

Liver: The liver weighed 2,100 Gm. and was smooth, greenish brown and of average firmness. The cut surface was brownish gray. The gallbladder and bile ducts were normal.

Kidneys: The kidneys weighed 320 and 210 Gm., respectively. The capsule stripped easily from a smooth surface devoid of hemorrhages. The cortex was 8 mm. in thickness and was greenish brown throughout. The pyramids were congested and the pelves normal.

Stomach: Marked generalized congestion of the gastric mucosa was present.

Ileum: Small hemorrhagic areas were noted in the mucosa of the lower part of the ileum.

Esophagus: The mucosa of the greater portion was dark red and soft and presented a "chewed-up" appearance. There was a slightly greenish cast in places.

Gastrocnemius Muscle: The muscle was a uniform red.

Aorta: Slight atheroma was noted.

Brain: The weight was 1,460 Gm. The pia-arachnoid was faintly yellow.

The adrenals, pancreas, bladder, ureters, genital organs and vertebral marrow were normal.

A culture of blood from the heart produced no growth. No evidence of septicemia was found. The antemortem finding of Staphylococcus aureus was attributed to contamination.

Microscopic Examination.—Heart: Rarely, a single muscle fiber was vacuolated or broken up into longitudinal hyaline strands with loss of striations and was strikingly infiltrated with mononuclear cells (histiocytes) and leukocytes. An occasional group of plasma cells adjoining a venule was noted. Occasional slight scarring, chiefly in the neighboring vessels, was present.

Lung: Small patches of fresh hemorrhage into the alveoli were noted. The walls were crowded with polymorphonuclear leukocytes, which were present in moderate numbers in the bronchial walls. Mononuclears containing carbon were fairly numerous.

Spleen: No hemosiderin or appreciable phagocytosis of red cells was apparent. There was increase of the plasma cells and, to a slight degree, of the polymorphonuclear leukocytes. Small deposits of neutral fat were noted in the reticular cells of the malpighian corpuscles.

Liver: There was no biliary stasis. Occasional small droplets of bile were observed in cells in the center of the lobule. The nuclei were often swollen. Binucleate cells were common. Many mitoses, multiple in a few instances, were noted. There was dense lymphocytic infiltration of some of the portal spaces without relation to the small bile ducts. The Kupffer cells often contained bile pigment and occasionally a small droplet of fat. Practically no fat was present in the hepatic cells. The perisinusoidal lymph spaces were usually wide and clearly defined.

Pancreas: Inspissated secretion and occasional polymorphonuclear leukocytes were observed in the dilated acini in scattered areas. Necrosis occurred in the center of one lobule, with sparse infiltration of the stroma by polymorphonuclear leukocytes.

Kidney: Marked interstitial infiltration, for the most part in the medulla, both diffuse and in foci, was chiefly of plasma cells, with occasional eosinophils, polymorphonuclear leukocytes or mononuclear cells. Two well developed follicles of small lymphocytes were noted. The tubules in this region contained considerable cellular débris and red granular material and occasionally a few polymorphonuclear leukocytes or mononuclear cells filled with dark bile pigment. Some tubules were much dilated, with evidence of regeneration of epithelium. Perhaps a fourth of the convoluted tubules showed swelling of the epithelium with narrowing of the lumen. A negligible amount of fat was present, but only in extremely finely divided form. The glomeruli were normal.

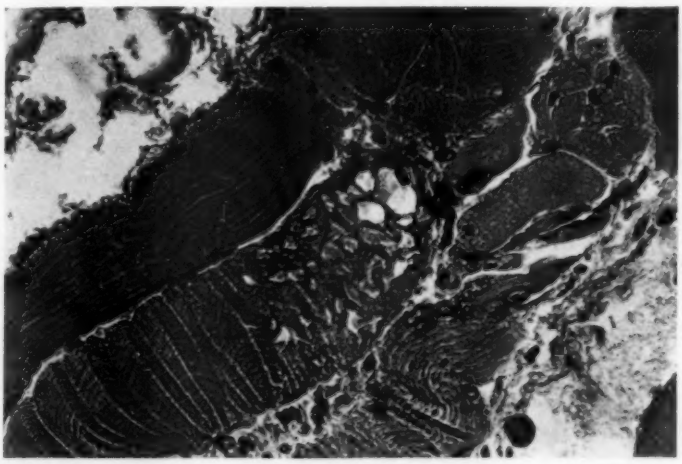

Fig. 1.—Photomicrograph of gastrocnemius muscle, showing the characteristic selective involvement of fibers and the marked vacuolation of one.

Adrenals: The cortical cells appeared depleted of lipoid droplets, but a stain for fat showed a fair amount present in finely divided form.

Vertebral Marrow: The marrow of the vertebrae was normal. No hemosiderin or increased phagocytosis of the red cells was noted. Megakaryocytes were numerous.

Esophagus: A massive exudate of blood, polymorphonuclear leukocytes and fibrin into and beneath the squamous epithelium was observed. In places the cells were lifted up and teased apart as though by fluid. Masses of cocci and other organisms occurred at the surface. The submucosa was edematous; the outer part was infiltrated and the vessels were crowded with polymorphonuclear cells. Several large isolated foci of lymphocytes were noted in the deeper portion.

Gastrocnemius Muscle (fig. 1): Necrosis of one or two or three adjoining fibers and local accumulation of mononuclear and plasma cells and occasional polymorphonuclear leukocytes were noted. The degenerated fiber was broken up into a few large, round hyaline masses, with slightly basophilic multinucleate remnants

of sarcolemma, often triangular, between them or at one side. Marked vacuolation of fibers at the margin of the area of degeneration was present.

Stomach, Brain and Choroid Plexus: The results of examination were negative. Levaditi stains were made of tissue from the kidney, liver, spleen, gastrocnemius muscle and lung. Spirochetes were seen only in the convoluted tubules of the kidney. From about six to a dozen organisms were present in a tubule, but only in three or four tubules in a section. A few appeared to be just within the cytoplasm of the epithelial cells, but there was no corresponding local reaction or necrosis (fig. 2).

A few suggestive forms were present in the muscle, occurring singly in the stroma or as groups of irregular rods (as though fragmented) within degenerating fibers. None were in or near the vacuoles.

Diagnosis: The final diagnosis in this case was established post mortem by the characteristic appearance of the liver, kidney and gastrocnemius muscle and

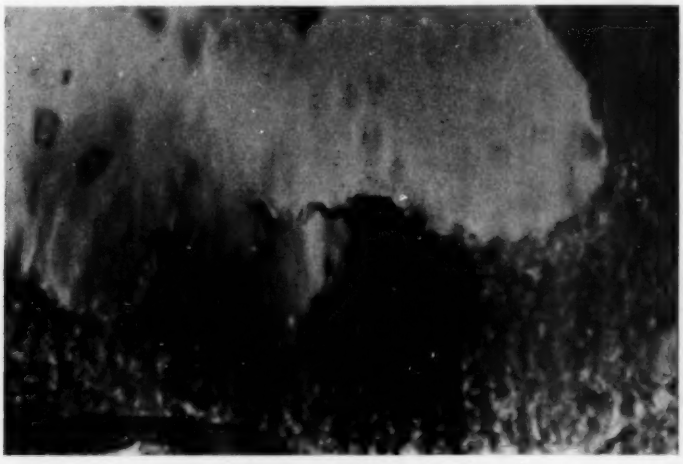

Fig. 2.—Spirochete in a tubule of the kidney. Two others lie at the left, apparently within the epithelium. Levaditi stain; oil immersion lens.

by demonstration of the spirochetes in the kidney. Inoculation of a guinea-pig and culture of the organisms unfortunately were not done.

Epidemiological Survey.—The patient was employed as fish cutter at a local plant known to be infested with rats. The fish (chiefly haddock and small cod) were brought in and the refuse removed on wooden conveyers. The cutting and filleting were done at fairly clean, but necessarily slimy, wooden benches. The floor of concrete was poorly drained and covered with dirty water and slime. The men wore rubber boots and gloves but were subject to cracks and slight cuts on the hands. The room was cleaned regularly once a week.

No cases of a similar nature could be recalled by any of the employees interviewed, and the physician who had charge of patients from this company and similar companies, as well as of many sewer workers and longshoremen, had no record of a similar case. Since this company doctor cared only for traumatic disabilities his statement meant little. Systemic disorders were treated by the personal physician of the employee. The living premises of the patient were not unusual. No other cases were reported either in the family or in the neighborhood.

The following procedures are advised in autopsies in suspected cases:

1. Examination by dark field of fresh scrapings from the kidney.

2. Histologic examination of (*a*) the skin (to check further the observations of Pick [14]), (*b*) the diaphragm, in cases in which hiccup was prominent, (*c*) the muscles of the eye if pain behind the eyes was a prominent feature, (*d*) the tonsils, pharynx, esophagus or other possible ports of entry showing signs of inflammation and (*e*) lymph nodes in the corresponding region which are enlarged.

3. Fixation of all tissues to be examined for spirochetes in a solution of formaldehyde, which is the proper fixative when the Levaditi stain is to be made.

THE INFECTING ORGANISM

Leptospira icterohaemorrhagiae is a true spirochete, with a central axial filament.[15] It is from 6 to 15 microns long and approximately 0.2 micron wide, tending to be smaller in human subjects than in guinea-pigs. Fine, tightly wound spirals enclose the axial filament in the stiffer middle part and are prolonged beyond it into straight, curved or hook-shaped ends, which are of greater mobility.

The organism is rapidly motile and moves in a straight line by spinning vigorously on its axis, one end being curved and perhaps acting as a propeller while the middle part and the other end remain straight. Motion is in the direction of the straight end. Division takes place by longitudinal fission. It differs from the treponemes in being insoluble in a 10 per cent solution of saponin.[16] Specific antiserum or a solution of sodium taurocholate produces lysis, progressing from the ends toward the middle portion.

EPIDEMIOLOGY

Nonpathogenic saprophytic spirochetes morphologically similar to that of Weil's disease occur in natural waters all over the world. They were first described in 1914 by Wolbach and Binger,[17] who discovered them in stagnant water obtained from a pond near Boston. They have since been found in mud of lakes and rivers, in garden soil, among decayed leaves, in puddles, surface-water and ditch-water, in sewers and reservoirs, in salt springs and sea water and in the tap water of many European cities,[15] as well as in 87.5 per cent of samples of municipal

14. Pick, L.: Berl. klin. Wchnschr. **54**:451 and 481, 1917.

15. Uhlenhuth, P., and Fromme, W.: Weilsche Krankheit, in Kolle, W.; Kraus, R., and Uhlenhuth, P.: Handbuch der pathogenen Mikroorganismen, Jena, Gustav Fischer, 1930, vol. 7, pt. 1, p. 487.

16. Noguchi, H.: J. Exper. Med. **27**:609, 1918.

17. Wolbach, S., and Binger, L.: J. M. Research **30**:23, 1914.

drinking water from forty-seven cities in the New England area.[18] These water spirochetes have all come to be referred to under the common specific name Leptospira biflexa. Actually, the group is composed of a great number of strains of close serologic relationship.[19] In addition to Leptospira (properly Spirochaeta) icterohaemorrhagiae of Weil's disease, pathogenic strains include: (1) Leptospira hebdomadis, the causative organism of akiyami or seven day fever in Japan; (2) Leptospira grippo-typhosa, which causes the swamp fever of eastern Europe, and (3) Leptospira canicola, which produces a disorder in dogs resembling Weil's disease.

Weil's disease itself has been reported from all over the world, and Schüffner determined the approximate serologic identity of original strains from the Netherlands, Berlin, London, Paris, Japan, the East Indies, Lisbon, Hamburg and New York (the strains of Noguchi). Serums from Greece, Copenhagen and the Belgian Congo also corresponded, an observation leading to the conclusion that a universal specificity exists.[10]

The virulent Weil strain has never been found except in places accessible to or infested by wild rats. These animals carry the organisms in the tubules of the kidney, excreting them in the urine and thus inoculating water, soil or food with which they come in contact. Rats from all over the world have been examined, and the spirochete was found to be present in roughly 10 per cent.

The following percentages for frequency of infestation among the rats examined in American cities were determined: Chicago, 3; Baltimore, 7 and 52; Washington, 10; Nashville, 10, and New York, 17 and 22.[15] In the Netherlands 45 per cent of adult rats, but only 3 per cent of young ones, carried the spirochete.[10] Both Norwegian and black rats, as well as mice, are implicated. They presumably pick up the spirochete by eating infected material. Feeding experiments have shown in a few instances that they can be infected by this route. Rat-to-rat infection may occur in cages.[20] Men have been infected by rat bite in at least 2 instances.[21]

Dogs may act as vectors, since they are subject to infection by the true Weil strain as well as by L. canicola.[10] No case of human infection from a dog due to a human strain has been reported. However, Schüffner was able to isolate L. canicola from the blood of a man suffering from an infection resembling Weil's disease, and he definitely proved that this strain could produce in man a disease simulating true

18. Dimitroff, V. T.: J. Infect. Dis. **40**:508, 1927.

19. Uhlenhuth, P., and Zuelzer, M.: Klin. Wchnschr. **1**:2124, 1922. Baermann, G., and Zuelzer, M.: Zentralbl. f. Bakt. (Abt. 1) **105**:345, 1928.

20. Uhlenhuth, P.: München. med. Wchnschr. **77**:2047 and 2098, 1930.

21. Ido, Y.; Hoki, R.; Ito, H., and Wani, H.: J. Exper. Med. **26**:341, 1917.

Weil's disease.[10] Certain flies (Haematopota and Stomoxys), lice and horse-leeches have been shown capable of transmitting the infection experimentally but are thought to be of no importance under natural conditions.[15] Nonvirulent spirochetes were found in the urine of 2 persons exposed to patients with Weil's disease and their excreta,[22] but there is little evidence that man may act as a carrier.

Dissemination by water occurred in a unique epidemic in Lisbon in 1931.[23] Drinking water obtained at a public fountain was responsible. Rat feces were found in the underground aqueduct.

Among the places in which human beings have contracted the disease are damp mines, rice-fields flooded with fecally contaminated water, sewers, canals, drainage ditches, stagnant moats, trenches (in war time), garbage pits, piggeries, breweries, slaughter-houses, moist soil or water containing decaying matter and public baths, where the hazard from rats is great and the water but slowly changed. Alkalinity of the soil or water is favorable to the organisms; salinity is unfavorable.[10]

Whether or not virulent leptospiras may occur in regions free from rats (if such places exist) remains to be determined. Buchanan[24] recovered them from the roof slime of a mine in a situation which appeared inaccessible to rats, but other parts of the mine were infested. Whether or not the common saprophytic form can undergo mutation or acquire increased virulence in the presence of abundant decaying matter or in the animal kidney is a question. Such an apparent transformation of a nonpathogenic strain into a virulent form took place during prolonged cultivation on serum medium and, in another instance, by repeated passage through guinea-pigs.[20] For all purposes, however, one may say: No rats, no virulence.

Incidence.—Extensive epidemics of jaundice occurred among Napoleon's soldiers in Egypt, during the American Civil War and in the Boer war. It is not determinable whether these cases represent instances of infectious jaundice or instances of Weil's disease. After the discovery of the causative organism, verified cases of Weil's disease were reported during the World War on the Italian front and in the British, German and French armies.

In recent years its occurrence has been chiefly among (1) persons working in infested places as previously enumerated, i. e., miners, sewer workers, slaughter-house workers, butchers, sluice or drain cleaners, bargemen and fishermen; (2) persons who have fallen into polluted waters accidentally or with suicidal intent, and (3) bathers and swim-

22. Frugoni, C., and Capellani, S.: Riforma med. **33**:439, 1917; quoted by Uhlenhuth and Fromme.[15]

23. Jorge, R.: Bull. Office internat. d'hyg. pub. **24**:88, 1932.

24. Buchanan, G.: Spirochaetal Jaundice, Medical Research Council, Spec. Rep. Ser. no. 113, London, His Majesty's Stationery Office, 1927.

mers. Between 1924 and 1933 in the Netherlands there were 452 cases, the incidence varying from 22 to 56 for every 400,000 in the three more densely populated coastal provinces.[10] In the city of Dordrecht 30 cases were traced to occupational circumstances, 44 to "water accidents" and 128 to bathing and swimming. The incidence of cases due to water accidents (1 case for every 75 accidents) was notably greater than the highest figure found for any bathing place (about 1 for every 4,200 baths). The greatest prevalence of infection was in the late summer (from July to October), but cases occurred in all months of the year. Statistics for other countries, where laboratory tests for aid in diagnosis were commonly made, are similar.

Port of Entry of Organism.—Guinea-pigs have been infected by rubbing the spirochetes into the skin.[2] Pigs with slightly scarified skin acquired the disease on being made to swim in water from heavily infected ditches.[25] Definite infection through cuts and needle pricks has been occasionally reported.[26] It is probable that a considerable number of human infections through the skin have occurred among barefooted workers in rice-fields, bathers, butchers and sewer workers with cracks, cuts or scratches on the feet, hands or elsewhere. Local enlargement of the lymph nodes in certain cases favors this view.[27]

Infection per os has been accomplished experimentally by a few investigators in both rats and guinea-pigs, although most attempts have been unsuccessful. The acid of the stomach and the bile in the duodenum exert a lethal effect on the organisms,[15] and the buccal and the pharyngeal mucosa appear a more probable port of entry than the gastro-intestinal tract, although Inada favored the latter route.[2] The greater frequency among victims of water accidents as compared with that among bathers has been attributed to unintentional swallowing or inhaling of water by the former persons.[10] The epidemic in Lisbon arising from drinking water is the outstanding example of infection by the oral route,[23] and certainly in the majority of situations conveyance of infected material to the mouth by the hands would be difficult to rule out.

Accidental laboratory infection occurred in 2 workers who were sprayed in the eyes, 1 with highly infected guinea-pig blood[28] and the other with a pure culture of a virulent strain of Leptospira. Marked injection of the conjunctival vessels is a regular and early symptom

25. Appelman, J. M.: Het isoleeren van Leptospira icterohaemorrhagiae uit water, Leyden, N. V. Leidsche Drukkerij, Morschsingel, 1934; abstr., Trop. Dis. Bull. **31**:514, 1934.

26. Wadsworth and associates.[4] Schüffner.[10]

27. Drew, J. G.: Brit. M. J. **2**:1142, 1934. Inada and others.[2]

28. Goebel, W.: Med. Klin. **12**:381. 1916.

in both men and animals, but it has nothing to do with the primary site of infection.

There has been 1 case of alleged transmission from husband to wife by cohabitation.[29]

Excretion.—In the guinea-pig spirochetes are excreted in large numbers in the urine, bile and bronchial secretions.[30] In human patients they are excreted in the urine; they have also been reported in the feces [2] and in isolated instances in the sputum [2] and vomitus.[31] Their presence in blood from menstruation or epistaxis is to be expected. Supposed infection from the urine has been observed in only 1 instance.[15]

Period of Incubation.—According to Inada, the period of incubation is from five to seven days. In 37 cases of infection due to water accidents in which the interval was accurately fixed Schüffner found it to vary between four and nineteen days, usually from seven to thirteen days, with an average of 10.3 days.[10]

Prevention.—Obvious measures include elimination of rats, removal of decaying matter and sources of pollution of water and chlorination of the water supply. Inada [32] was able to reduce substantially the incidence in the coal mines by pumping out the water. He recommended drainage and application of lime to infected soil. Eminently successful results in the rice-fields were obtained by Tohyama.[31] He found calcium cyanamide to be the only substance which would kill the spirochete and at the same time fertilize the rice; he determined that application of the equivalent of 4.17 pounds (1,891.5 Gm.) per acre (4,046.87 square meters) provided a sufficient concentration to accomplish the former result.

Persons coming into contact with patients should be advised of the possibility of contagion. All dejecta must be carefully removed and destroyed. The urine of a convalescent patient may be examined after forty days to determine whether he has become a carrier.

Prophylaxis.—Active immunization by means of vaccination has strikingly lowered the incidence in many instances. In certain regions of Japan there were but 5 cases per month where there had been from 15 to 30 before.[32] Wani vaccinated 10,368 persons in six coal mines, with a resulting morbidity rate of 0.12 per cent, or less than one-ninth that among unvaccinated persons in another mine.[33] Baermann found no cases among 80 vaccinated men, although they continued

29. Doeleman, quoted by Schüffner.[10]

30. Clément, P., and Fiessinger, N.: Bull. et mém. Soc. méd. d. hôp. de Paris **40**:2073, 1916.

31. Tohyama, Y.: Japan M. World **4**:193, 1924.

32. Inada, R.: Japan M. World **2**:189, 1922.

33. Wani, H., quoted by Inada.[32]

to work in an infected district.[34] According to Noguchi, the vaccine to be effective should contain 200,000,000 organisms per cubic centimeter. Inada used organisms grown on Noguchi's medium and was able to immunize guinea-pigs with 0.01 cc. Baermann used a mixture of about 116 strains; the organisms were killed by heating to 65 C. and a 0.5 per cent solution of phenol was added.[15] Wani gave doses of 2 and of 3 cc. subcutaneously one week apart. He was able to demonstrate spirocheticidal properties of the blood as late as nineteen months after vaccination. Baermann used a single dose of 5 cc. intramuscularly or doses of 1 and 2 cc. from five to eight days apart.[35]

Because of the rarity of the disease in this country active immunization on a large scale would not be practicable. It is strongly advised, however, for persons in certain occupations, including sewer workers and bacteriologists known to be exposed to infection.

Immunity.—Although hens and frogs possess antispirochetal substances in their blood, there is no natural immunity in man.[15] A powerful active immunity, however, is acquired by an attack of the disease. Only 1 case of a second attack in the same person has ever been reported.[35a] This immunity is at least partly due to antibodies which appear in the blood between the seventh and the fifteenth day of illness.[36] They attain their highest concentration during convalescence and may persist for many years. Postmus [37] found these antibodies well developed even after eight years or longer. In 1 person they were present after twenty-two years.[15] In a patient receiving serum during an attack the resulting immunity may be lower than that of untreated patients. Schüffner cited the case of a treated patient the titer of whose serum was 1 : 3,000, whereas without serum treatment the titer is commonly 1 : 30,000.

Passive immunity is successfully conferred by administration of either convalescent serum or immune horse serum. Inada and his associates [36] were able to protect guinea-pigs with serum from convalescent patients or from immunized goats.[38] They also immunized horses, and with antiserum given to patients admitted before the seventh day of the disease they were able to reduce the mortality rate in their severest cases from 57.1 to 38.5 per cent. Of 21 patients under the

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34. Baermann, G., and Smits, E.: Zentralbl. f. Bakt. (Abt. 1) **105**:368, 1928.

35. (*a*) Baermann, G.: Die kurzfristigen Spirochätenfieber, in Kolle, W.; Kraus, R., and Uhlenhuth, P.: Handbuch der pathogenen Mikroorganismen, Jena, Gustav Fischer, 1930, vol. 7, pt. 1, p. 661. (*b*) Uhlenhuth and Fromme.[15]

36. Inada, R.; Ido, Y.; Hoki, R.; Ito, H., and Wani, H.: J. Exper. Med. **24**:485, 1916.

37. Postmus, S.: Nederl. tijdschr. v. geneesk. **4**:2648, 1933.

38. Donkeys, sheep or rabbits are also suitable.

age of 40 only 1 died. A rapid disappearance of spirochetes from the blood was observed.

MORBID ANATOMY

The clinical effects of this disease are those of a severe generalized infection of the blood stream. The early extreme prostration is perhaps the most striking manifestation. Accompanying organic changes are somewhat variable and may be relatively slight.

The usual observations at autopsy are generalized jaundice and lesions characteristically involving the following structures: (1) capillaries, (2) liver, (3) kidneys and (4) skeletal muscle.

1. Damage to the capillaries is manifested by minute hemorrhages generally distributed in the body. They are most common under the peritoneum and pleura and in the gastro-intestinal tract, kidneys, adrenals, nasal mucosa and skin. They may also occur beneath the endocardium or pericardium, under the capsule or within the portal spaces of the liver, in the mesentery, spleen or pancreas, beneath the mucosa of the larynx and trachea, in the lungs and in the mucosa of the bladder.

The brain may contain pea-sized hemorrhages [39] or the tentorium and dura may be involved.[40] Miller noted hemorrhages in the tibial nerve.[39]

These hemorrhages are considered to be the result of a local toxic effect of the spirochete on the wall of the vessel. Diapedesis of red cells through the unbroken wall was observed in the lung by Beitzke.[40] Hemorrhages are of considerable importance in the severer cases, accounting for the epistaxis, hematuria, hematemesis, melena, hemoptysis or purpura which is sometimes present. Death may even result from gastro-intestinal hemorrhage.[14]

2. The liver may appear normal on gross examination. It is commonly slightly enlarged and often bile-stained,[41] never shrunken as in acute yellow atrophy. Obstruction of intrahepatic or extrahepatic bile ducts is lacking. (Plugs of mucus and epithelium in the lower end of the common duct have been described only in rare instances.[42])

Microscopically, two significant changes occur with fair regularity: proliferation of hepatic cells and traces of biliary stasis in the central part of the lobule. The hepatic cells show active division, which may be predominantly amitotic.[43] The nuclei are often swollen, and there

39. Miller, J. W.: München. med. Wchnschr. **64**:1572, 1917.

40. Beitzke, H.: Berl. klin. Wchnschr. **53**:188, 1916.

41. Garnier, M., and Reilly, J.: Arch. de méd. expér. et d'anat. path. **28**:228, 1918.

42. Oberndorfer, J.: München. med. Wchnschr. **65**:1190, 1918.

43. Verne, J.; Bariéty, M., and Albeaux-Fernet, M: Ann. d'anat. path. **9**:200, 1932.

are sometimes two or three in a cell. Mitoses may be frequent, however. Cloudy swelling, loosening or dissociation of the cells as though from edema and widening of the perisinusoidal lymph spaces have been described.[13b] Fatty infiltration is absent or slight. Retained bile usually occurs in small amount centrally in the lobule within a few bile capillaries or as small droplets or granules in the cytoplasm of the cells; there is none in the periphery of the lobule, nor is there evidence of stasis in the interlobular or larger bile ducts.

Other changes include swelling and homogeneization of the chrondriome,[43] deposition of fat in the Kupffer cells, infiltration of the portal spaces by lymphocytes and a few polymorphonuclear leukocytes or eosinophils.

In some cases the liver has shown varying degrees of necrosis, usually slight and focal but occasionally so extensive as to simulate that of acute yellow atrophy. There is no marked reduction in size, however. In 1 patient, dying on the twelfth day, the liver was grayish brown, with the centers of the lobules dark red, as seen on the cut surface. It weighed 1,475 Gm.[44] The diagnosis was confirmed by concomitant myositis. Such conditions are regarded simply as the result of an unusually severe infection.

How will one account for the jaundice? There are two explanations: (*a*) that swelling of the cells and edema (Beitzke's "toxic edema") produce mechanical obstruction by compression of the bile capillaries and (*b*) that the hepatic cells are so damaged by the spirochetal toxin that their bile-excreting function is crippled. Probably both factors are involved. Their relative importance could be better estimated if tissue could be studied from the earliest days of the disease. At all events, the stimulus which produced the rather uniform, diffuse, regeneration-like appearance in the later stage may conceivably have arisen from an early generalized toxic influence insufficient to cause actual necrosis. Excessive hemolysis is apparently not a feature. Increased phagocytosis of red cells in the reticulo-endothelial system is only inconstantly observed.[40] Fragility of the red cells is not increased.[13b]

3. The kidneys are often enlarged and usually show (*a*) the greenish-brown stain common to bile-saturated organs in any case of jaundice, (*b*) swelling and more or less marked necrosis of the epithelium of the convoluted tubules and (*c*) interstitial infiltration consisting chiefly of lymphocytes, with smaller numbers of polymorphonuclear leukocytes and eosinophils.[40] There may be small hemorrhages under the capsule, into the interstitial tissue or into the tubules. Granular and bile-containing casts are common. Little fat is present. The glomeruli are unchanged

44. Bates, J. E.: Canad. M. A. J. **16**:1466, 1926.

in most cases. The damage is thus predominantly in the tubules. According to Pick, it is toxic in origin and due to the spirochetes, not to the bile.

Other changes sometimes observed include swelling or proliferation of the epithelium of Bowman's capsule, crescent formation, acute hemorrhagic glomerulonephritis,[5a] acute nephritis resembling that of scarlet fever,[14] marked interstitial infiltration by polymorphonuclear leukocytes almost to the extent of abscess formation [45] and infiltration by great numbers of eosinophils.

The extent of the renal injury is often better indicated by the clinical and laboratory findings than by the histologic structure, as in the case reported in this paper.

4. The muscles most severely involved are those of the calf. Others, including the pectoral muscles, are similarly but less affected. The gross appearance is usually normal,[14] but there may be punctate hemorrhages or bile-stained foci of degeneration as large as from 4 to 5 mm. in diameter.

As seen microscopically, the process characteristically selects isolated fibers and only part of the fiber; with more extensive involvement adjacent fibers or even a whole field are concerned. There are vacuolation, swelling, loss of striations, hyalinization, infiltration with histiocytes, polymorphonuclear leukocytes and plasma cells, breaking up of the substance of the fiber into large, round lumps of hyaline material, resorption and proliferation of the nuclei of the sarcolemma. Hemorrhage into the empty sheath has been observed.

The picture differs strongly from that of Zenker's degeneration, such as occurs in typhoid fever. The latter form may usually be detected macroscopically, involves chiefly the adductor and abdominal muscles, commonly gives rise to hematoma, affects all the fibers in large areas and causes swelling of the fibers without resorption and shrinkage.

The lungs are sometimes normal. Congestion and edema may be marked. The pleural surface may be strikingly spotted with hemorrhages.[14] Confluent patches of hemorrhage in the parenchyma resemble infarcts.[45] There may be miliary pneumonic foci.[15] The accompanying bronchial secretion is sanguinopurulent.[39] The gross appearance of the lung is more characteristic in the guinea-pig.

The heart may show scanty cellular infiltration around the small vessels [40] or in the myocardial stroma. In 1 case there were large areas of degeneration of the muscle fibers of the type seen in skeletal muscle; i. e., vacuolation, breaking up into lumps and proliferation of the sarcolemma.[46] Fibrinous pericarditis was noted in 1 instance by Pick.

45. Hart, C.: Berl. klin. Wchnschr. **54**:285, 1917.

46. Reinhardt: Med. Klin. **13**:981, 1917; abstr., München. med. Wchnschr. **64**:1403, 1917.

Miller [39] observed a large area of infarct-like necrosis in the wall of the left ventricle with hemorrhage and local pericarditis. Drägert [47] reported 2 cases of vegetative endocarditis and demonstrated the spirochete in the vegetation, which was on the interventricular septum 2 cm. below the aortic valve in 1 case and at one of the commissures of the valve in the other.

The spleen is usually not enlarged and is of moderately firm consistency. Hemorrhages, deposits of hemosiderin and numbers of phagocytes with ingested red cells are occasionally seen.[24]

The gastric mucosa is commonly diffusely peppered with petechial spots; serious hemorrhage may arise in this area or from points in the small or the large bowel. The duodenal mucosa may be swollen and bluish red [13c] and the wall hemorrhagic.[48] Catarrhal inflammation may occur in the duodenum and terminal portion of the ileum.[49] Petechial spots under the serosa are common throughout the tract. In 1 case of colitis, foci of hemorrhage and necrosis [47] were described. How great a part is played by uremia in the production of these lesions has not been determined.

The pancreas was hemorrhagic throughout in 2 of Mayer's cases; hemorrhages into the wall of the duodenum and the ampulla also were present.[48] Flabby consistency of the organ and small size of the cells were noted; [40] marked edema was noted in 1 case and foci of necrobiosis in another.[39]

The adrenals fairly constantly show hemorrhage. In 1 case the whole organ was involved, the hemorrhage apparently starting in the medulla.[13b] In another case the picture of total hemorrhagic infarction was presented.[48]

Lymph nodes were occasionally seen to be enlarged in the upper part of the cervical region only, by several investigators.[50] Swelling of local nodes was noted by Japanese investigators in cases of infection supposedly through the skin.[27]

In the tonsils Miller noted superficial acute inflammation with formation of vesicles in the region of the crypts, sometimes spreading to surrounding structures.[39] This condition was present in 4 of 7 of his cases but has not been observed by other investigators.[51]

The pharynx was the site of acute fibrinous inflammation in 2 cases.[14] In another instance the pharyngeal muscle was severely

47. Drägert, E.: Virchows Arch. f. path. Anat. **292**:452, 1934.

48. Mayer, A.: Deutsche med. Wchnschr. **44**:857, 1918.

49. Kaneko, R.: Ueber die pathologische Anatomie der Spirochaetosis icterohaemorrhagica Inada, Vienna, Rikola Verlag, 1922.

50. Hecker and Otto: Deutsche med. Wchnschr. **18**:820, 1911. Beitzke.[46]

51. Drägert.[47] Beitzke.[40]

damaged.[47] The common occurrence of dysphagia suggests a greater frequency of lesions in this region.

The bone marrow is unchanged.[47]

The skin, either in the presence or in the absence of rash, shows cellular infiltration around the arterioles and capillaries of the cutis, following them into the papillae.[14] Lymphocytes and plasma cells but few polymorphonuclear leukocytes are present; in the epidermis wandering polymorphonuclear leukocytes or nests of them may occur beneath the horny layer. These observations are not constant, however.[15]

Spirochetes are distributed in the blood to every part of the body. They have been observed in all the organs as well as the walls of arteries, muscles, lymph nodes, nervous system and internal ear. Whereas in the guinea-pig they are extraordinarily abundant in the organs at the time of death, in man they are usually scarce. Besides the liver and kidneys, the organs and tissues show the spirochetes in the following order of frequency: adrenals, myocardium, intestinal wall, appendix, pancreas, prostate, lung, spleen, lymph nodes, skeletal muscle and wall of the bladder.[52] In the liver they may be seen in the interstitial tissue and the Kupffer cells, and in the later stages chiefly within the hepatic cells. Their scarcity may be indicated by the result of one investigation in which long and exhaustive search yielded only 1 organism in sections of the liver taken from 5 patients and only 2 spirochetes in sections of muscle from 2 patients.[40] In the kidney, on the other hand, they occur with considerable regularity in the lumen or within the epithelium of the convoluted tubules and persist there after their disappearance from all the other organs.

A considerable variety of incidental conditions have been observed in cases of Weil's disease. Many of them appear irrelevant. Other complications, such as tonsillitis, bronchitis, pharyngitis, esophagitis or catarrhal enteritis, might conceivably have something to do with the original port of entry of the spirochetes. It is more likely, however, that these inflammatory conditions are purely secondary lesions with more or less marked superimposed bacterial infection. Demonstration of the primary point of penetration in the absence of trauma remains a challenge to the pathologist.

CLINICAL PICTURE

The recognized concept of Weil's disease in foreign countries has been well described by Valassopoulo,[13a] Dawson,[13b] Inada,[53] Uhlenhuth,[15] Schüffner[20] and many other investigators. It has been customary to divide the disease into three stages, each with its characteristic features in regard to the behavior of the spirochetes in the blood, the anti-

52. Kaneko, R., and Okuda, K.: J. Exper. Med. **26**:325, 1917.
53. Inada, R.: J. Exper. Med. **26**:355, 1917.

bodies and the excretion of the organisms in the urine, as well as in respect to certain clinical signs and symptoms. A knowledge of these features is essential if one wishes to apply correctly the various diagnostic procedures. It is important to remember that in from 10 to 20 per cent of the cases the disease is so mild that the patient is hardly forced to bed.[10] Before the use of laboratory tests as a routine these mild cases were never recognized as instances of Weil's disease.

First or Febrile Stage.—This stage is characterized by free circulation of spirochetes in the peripheral blood, lack of antibodies and absence of the organisms in the urine.

The disease is ushered in usually with a severe chill, followed by sustained high fever (102 to 104 F.). Then occur severe headache (often with pain behind the eyes), muscular pains (usually in the legs and back) and marked prostration. In some of the severer cases the prostration may be out of proportion to the rest of the symptoms and dominate the clinical picture. Symptoms referable to the gastrointestinal tract may include anorexia, dysphagia, abdominal pain, nausea, vomiting and diarrhea. Occasionally there is cough or hiccup. Herpes labialis which becomes hemorrhagic is usually present. Varying cutaneous eruptions have been reported. Conjunctivitis is often striking and has been commented on by numerous writers as characteristic and important. Signs of meningeal irritation occur if the spirochetes enter the cerebrospinal fluid. The white cell count is elevated, but only rarely is it more than 20,000 per cubic millimeter. This stage lasts from four to seven days.

Second or Toxic Stage.—(This term has been substituted for the usual name "icteric stage," in order to correct the impression that jaundice is a necessary feature.) This stage is characterized by lack of spirochetes in the blood, development of antibodies and excretion of many organisms in the urine. Renal and hemorrhagic tendencies may be mild or absent in the cases in which jaundice is not shown.

Jaundice appears in 50 per cent of the cases and gradually deepens. The icteric index may reach 150 or more. A positive reaction to the direct van den Bergh test is expected, because hepatitis is largely responsible for the jaundice. An important triad of physical signs, particularly in cases of jaundice, is enlargement of the liver associated with absence of a palpable spleen and of generalized adenopathy. In rare instances swelling of the lymph nodes draining the site of the portal of entry of the organism may occur. Schüffner stressed the important prognostic dictum that among the groups of patients without jaundice there is practically no mortality. Most of the symptoms of the first stage decrease in intensity, and the fever falls by lysis. Hemorrhagic diatheses appear in the majority of cases (being more severe when jaundice is present) and may manifest themselves as

petechiae, epistaxis, subconjunctival hemorrhage, bleeding from the gums, hematuria, hemoptysis, melena or hematemesis. The patient becomes weaker and the heart action more feeble. Oliguria, with albumin, casts or red cells in the urine, is almost constantly present in the cases of more severe disease. The nonprotein nitrogen content of the blood is usually elevated and in fatal cases may reach 200 mg. per hundred cubic centimeters. Anuria is present in many fatal cases. Death occurs in this stage and may be due to toxemia, cardiac insufficiency, renal failure with uremia, pulmonary edema or hemorrhage. The mortality rate ranges from about 5 per cent in Europe to as high as 50 per cent in Japan. The second stage lasts from seven to ten days.

Third or Convalescent Stage.—This stage is characterized by the disappearance of spirochetes from the blood, their abundant excretion in the urine and complete development of antibodies in the blood.

The stage begins in the third week and is marked by gradual subsidence of all the earlier symptoms. Anemia and marked emaciation are commonly seen in the cases of severe infection and may last for months. A second fever, known as "after-fever," which lasts from four to twenty days, is seen in from about 25 to 40 per cent of the cases and starts usually during the third week of the disease. This fever was believed by Inada to be due to disintegrating toxins during the height of serologic immunity; he based this opinion on the fact that at the time of the fever there is no return of the principal symptoms and the blood is not infectious.

DIAGNOSIS BASED ON LABORATORY DATA

The blood or urine is examined according to the stage of the disease, it being remembered that the blood contains spirochetes during the first week only, or occasionally as late as the ninth day, and that the urine does not contain the organisms until about the tenth day, from which time they persist until as late as the thirtieth day. They have also been found in the cerebrospinal fluid during the first, and occasionally during the second, week.[54]

Because serum treatment may be highly successful in the early stage and greatly diminished in effectiveness if delayed until the jaundice is fully developed,[54] early diagnosis is desirable. The patient may die during the time required for the disease to develop in an inoculated guinea-pig. Hence search for the organisms in the blood should be made at once. If this is unsuccessful, other tests must be utilized.

Examination of the Blood by Dark Field.—This method is rarely successful with whole blood. Centrifugation of the blood three times

54. Inada, R.; Ido, Y.; Hoki, R.; Ito, H., and Wani, H.: J. Exper. Med. **27**:283, 1918.

according to the method of Blanchard and Lefrou [55] increases the chances of success, yet often fails. It is difficult to throw down the spirochetes even at high speed. Good results may sometimes be obtained, however, simply by centrifugating at low speed sufficiently to precipitate the red corpuscles and examining a thick layer of the supernatant plasma. The spirochetes are said to be from ten to twenty times more readily detected by this means.[56]

Examination of the Urine by Dark Field.—After the tenth day this is often a successful method of diagnosis, but though the results are negative the evidence is not conclusive. The sediment from 50 cc. of freshly voided urine should be used.

Inoculation of Guinea-Pigs.—This means is the most satisfactory and is the usual method of establishing the diagnosis. What the mouse is to the pneumococcus, the guinea-pig is to the leptospira of Weil's disease. One injects intraperitoneally from 3 to 5 cc. of blood (more than this may kill the animal prematurely) and 5 cc. of cerebrospinal fluid or the sediment from 40 to 60 cc. of freshly voided urine suspended in 5 cc. of a physiologic solution of sodium chloride.

After a period of incubation of six days or more there is a sharp rise in temperature. As the peak is reached jaundice appears, and this is followed in about twenty-four hours by a fall in temperature, collapse and death. Necropsy reveals (1) jaundice of the skin and all the internal surfaces; (2) petechial hemorrhages in the skin, in the muscles of the abdomen and thigh, under the peritoneum and in the gastrointestinal mucosa; (3) multiple small, sharply defined hemorrhages in the lungs, especially through the lateral portion of the lower lobes, the spotted appearance of which suggests "the wing of a mottled butterfly;" [13b] (4) acute congestion of the kidneys with minute hemorrhages, and (5) large hemorrhages in the adrenals.

Spirochetes are readily demonstrated in dark field or india ink preparations of an emulsion of the liver and by impregnation of pieces of the kidney or liver by the Levaditi method. They may be cultivated or reinoculated into other guinea-pigs from the liver, kidney, urine or blood from the heart. Pigs may become ill of the disease and then recover. The pigs which do not die should therefore be killed and examined after from seven to ten days.[5b] Still earlier results may be obtained by examining the animal's peritoneal exudate removed from day to day with a capillary pipet.[15]

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55. Blanchard, M., and Lefrou, G.: Bull. Soc. path. exot. **15**:699, 1922.

56. Ruys, A.: Nederl. tijdschr. v. geneesk. **77**:3364, 1933; quoted by Schüffner.[10]

Culture of the Patient's Blood.—In the early stage the blood may be cultured according to the method of Manteufel.[57] To each of several tubes containing from 3 to 10 cc. of sterile distilled water from 2 to 3 cc. of the patient's blood is added, and the tubes are incubated three or four days at from 25 to 30 C. Examination by dark field in positive cases will then show spirochetes in at least one of three or four tubes. The organisms will live in this medium for three or four weeks.

Preparation of Culture of the Spirochetes for Stock Use.—This is best accomplished, according to Uhlenhuth and Fromme,[15] by using a liquid medium containing sterilized tap water and rabbit serum. Each of the narrow (1 cm.) tubes is filled with 2.5 cc. of water and 0.2 cc. of fresh serum; the mixture is then inactivated for one hour at 60 C. After inoculation with an emulsion of liver, blood from the heart or peritoneal exudate, paraffin is poured in, and the tubes are incubated at from 30 to 35 C. or less. The reaction must be neutral or slightly alkaline (the optimum p_H is 7.6; the maximum, 8.3). The use of hard water or saline solution is unfavorable. Growth begins in from twenty-four to forty-eight hours and attains a maximum in from five to ten days.[15] Transplantation should be made of weakly growing strains every six days; with vigorous growths, every three weeks will suffice. The medium used at present in the Netherlands was described by Davidson.[12] A similar medium was successfully used by Korthof.[58]

Agglutination Procedures.—Agglutination of known organisms with the patient's serum is not applicable as a diagnostic aid before from the sixth to the eighth day of the disease, at which time the antibodies first appear in the blood.[59] The test may be performed with living or killed cultures. Details of the technic used in the Netherlands were well described by Davidson.[12] Dilutions of serum of from 1:10 to 1:100,000 are made, an equal volume of spirochetal emulsion is added, and examination is made by dark field after incubation for three hours at 32 C.

The usual result with a serum giving a strongly positive reaction and living organisms is agglutination in dilutions as high as perhaps 1:300 and lysis (but no evident agglutination) in the higher dilutions (perhaps from 1:100 to 1:30,000). The use of a killed culture containing a 0.5 per cent solution of formaldehyde prevents lysis and permits agglutination in the highest dilution compatible with the strength of the serum (i. e., 1:30,000). There is also less danger in handling the culture.

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57. Manteufel, P.: Deutsche med. Wchnschr. **47**:461, 1921.
58. Korthof, G.: Zentralbl. f. Bakt. (Abt. 1) **125**:429, 1932.
59. Baermann, G., and Zuelzer, M.: Klin. Wchnschr. **6**:979, 1927.

According to Baermann,[35] the titer on the first day on which a positive result appears is already 1:100; it rises quickly in the next four to six days to 1:1,200 and in the course of the next ten to fourteen days to from 1:10,000 to 1:50,000 and higher. The height of the titer is independent of the severity of the illness, and the serums will almost always agglutinate at the same time other strains of Leptospira, often to an appreciably greater extent than the homologous one.

During the first fifty days after onset of the disease the agglutinating power is very high, and, in general, titers beween 1:10,000 and 1:50,000 (with an average of 1:34,000) are observed. The titer falls rapidly to about 1:700 during the next fifty days and then declines gradually, averaging 1:300 between the three hundredth and the nine hundredth day. The agglutinating power of the serum is entirely negative in about a third of the cases from the two hundredth day on.[35]

The agglutination test is useful in diagnosing cases in which previous infection is suspected as well as those of the acute state of the disease, and it is important to realize that a positive reading in dilutions of the lower hundredths during an acute illness stimulating Weil's disease may really be due to an old attack and may thus lead to false diagnosis. Gaehtgens reported an apparently false positive reading in a case of infection with the paratyphoid B bacillus which is perhaps to be explained on this basis.[60] A carefully determined history and failure of the titer to rise should reveal the true situation.

Agglutination with the dog strain (L. canicola) may advantageously be attempted, as well as examination of the urine of any dog suspected of being a vector. For specific agglutination of this kind Castellani's absorption test with a culture treated with formaldehyde is used.[61]

The Pfeiffer Phenomenon.—This test may be utilized for aid in diagnosis and for approximate titration of the patient's serum. One cubic centimeter of an emulsion of spirochetes mixed with an equal amount of serum is injected intraperitoneally into a guinea-pig weighing 200 Gm. (or less), and the peritoneal fluid is examined by dark field after one and one-half hours. The highest dilution of the serum which will still produce complete lysis of the organisms is subsequently determined. Tests may be carried out similarly to find the smallest amount of serum which will protect a guinea-pig from infection by 1 cc. of culture given intraperitoneally. (Inada found that 0.01 cc. of the horse serum which he used would accomplish this result and that it was as strong as convalescent serum.[36]) Unprotected guinea-pigs used as controls die within

60. Gaehtgens, W.: Klin. Wchnschr. **12**:697, 1933.

61. Castellani, A., and Chalmers, A.: Manual of Tropical Medicine, ed. 3, New York, William Wood & Company, 1919, p. 1397. Schüffner.[10]

the usual time (from five to twelve days). The adequately protected pigs survive this period, but some may die later (from the fourteenth to the nineteenth day).[62]

Complement-Fixation Test.—This procedure, with antigen made from a culture of the spirochetes, has been used with marked success by Gaehtgens.[60] With 70 unknown serums the results of the agglutination and complement-fixation tests coincided perfectly, 29 giving positive reactions. Higher titers were obtained by agglutination tests, but complement-fixation gave more clearcut negative readings in some cases in which agglutination was questionable in the lower dilutions. The test may be of special value in early diagnosis. The average titer in 5 cases on the seventh and eighth days of the disease was strongly positive (1:300). The test also constitutes a valuable confirmatory check on the results of agglutination.

Precipitation Test.—Hindle and White[63] isolated a specific soluble substance from the spirochetes which in solution gave precipitation in dilutions of the antiserum of the homologous spirochete. Details of this method have not to our knowledge been published.[64]

Adhesion Test.—Recently this method of diagnosis was proposed by Brown,[65] who stated that the reaction to this test was easier to read than the agglutination reaction and that the test could be performed more rapidly and was equally sensitive and specific. The adhesion test is based on the principle that particles, such as bacteria or blood platelets, become adherent to the spirochetes in the presence of immune serum. The details of the technic are well described in the paper just mentioned. The indications for its use are the same as those for the agglutination test.

COMMENT ON THE PRESENT CASE

The clinical history, physical findings and laboratory data were typical of Weil's disease. The occupation (fish cutting) should in itself have aroused suspicion. In consideration of the differential diagnosis Weil's disease was mentioned as a rare possibility. Unfortunately at that time the value of laboratory procedure was not appreciated, and a definite diagnosis was not made until after the necropsy. It is even conceivable that had an immediate diagnosis been made and serum been available the patient might have been saved.

The record of the necropsy in our case is the first complete pathologic study of an uncomplicated case of Weil's disease to be reported in this

62. Ido, Y.; Hoki, R.; Ito, H., and Wani, H.: J. Exper. Med. **24**:471, 1916.
63. Hindle, E., and White, P. B., in discussion on Schüffner.[10]
64. Lusena, M., and Cralinfanti, E.: Soc. internaz. di microbiol., Boll. d. sez. ital. **6**:77, 1934.
65. Brown, H. C.: Brit. M. J. **1**:411, 1935.

country. The American literature contains the results of three earlier postmortem examinations. McDowell [5a] limited his description to a few lines, while both of Ball's [9] cases were complicated by other diseases.

This pathologic study illustrates that remarkably normal histologic conditions in the liver are possible in a patient dying as early as the end of the first week. Absence of biliary stasis and lack of evidence of increased destruction of red cells in the spleen or bone marrow fail to throw further light on the mechanism of the production of jaundice.

The high content of nonprotein nitrogen (200 mg.) in the blood of this patient was unusual with so little apparent damage of the renal parenchyma, but the débris in the tubules was evidence of a not inconsiderable preexisting necrosis of epithelium. Hepatitis may have been responsible for the low content of cholesterol found in the blood (90 mg. per hundred cubic centimeters). The high value for phosphorus (13.6 mg) was checked by duplicate determination. We are unable to explain it.

Necrosis of certain isolated fibers in the heart is believed to be a specific result of the spirochetosis. Acute esophagitis has not to our knowledge been previously described in this disease.

COMMENT ON THE AMERICAN CASES

The available data on the reported American cases have been compiled in two tables. It can be readily seen that each case presents a clinical picture which, in spite of minor variations, resembles the classic European and Japanese types of the disease. With 1 exception (the accidental infection of a woman technician), all the cases occurred in men of middle age. In no instance was there an observed secondary case. The onset of the disease occurred during any season of the year. Occupations (not listed) were of interest in that the group of patients included a sewer worker, a member of a swimming team, a cook, a laborer and a fish cutter. Contact with rat-infested buildings was mentioned several times. Also in 1 instance the disease developed after the patient had been soaked in rain-water.

Jaundice and prostration occurred in all cases. This association is interesting in view of the fact that over one half of the European cases occurred without jaundice; it probably means that the nonicteric cases are not recognized in this country. Chills, fever, headache, muscular pain, vomiting and hemorrhagic tendencies were almost constantly present. Enlargement of the liver and absence of palpable spleen and lymph nodes were rather constant physical signs. Dysphagia, cough, hiccup, herpes, pharyngitis and conjunctivitis were minor signs and symptoms. In 83 per cent of the cases there were characteristic findings in the urine. When recorded, the nonprotein nitrogen content of the blood

was definitely elevated in all but 1 case, in which, curiously enough, no changes in the urine were shown.

Weil's disease being spirochetal, the Wassermann reaction might perhaps be expected to be influenced, but it was consistently negative.

TABLE 1.—*General Information and Laboratory Data in Cases of Weil's Disease Reported in the United States* *

Case	Author Reporting Case	Age, Years	Sex	Month of Occurrence of Disease	Laboratory Data: Non-protein Nitrogen, Mg.	Urine: Albumin	Urine: Red Cells	Urine: Casts	Wassermann Reaction	White Cell Count
1	Wadsworth	?	F	Feb.	—	0	0	0	Negative	+
2	McDowell	43	M	July	—	+	+	+	Negative	—
3	Sailer	51	M	Aug.	—	+	0	0	Negative	—
4	Sailer	44	M	Oct.	—	+	+	+	—	Normal
5	Hayman and Lynch	42	M	Oct.	73	+	0	+	Negative	10,300
6	Towler and Walker	31	M	Dec.	83	+	+	+	Negative	19,000
7	Mulholland and Bray	20	M	Feb.	—	+	0	+	Negative	14,200
8	Cushing	35	M	Sept.	46	+	0	0	Negative	10,400
9	Cushing	37	M	Oct.	30	0	0	0	Negative	11,600
10	Ball	51	M	Jan.	162	+	+	+	Negative	14,200
11	Ball	42	M	Nov.	—	+	0	+	Negative	9,000
12	Jeghers, Houghton and Foley	38	M	May	200	+	0	+	Negative	28,750

* In this table — indicates that data were not available; +, present or increased.

TABLE 2.—*Clinical Signs and Symptoms in Cases of Weil's Disease Reported in the United States* *

Signs and Symptoms	Case 1	2	3	4	5	6	7	8	9	10	11	12	Percentage of Positive Findings
Initial chill	?	?	+	+	+	+	0	+	+	?	+	+	65
Fever	+	+	+	+	+	0	+	+	+	+	+	+	91
Headache	0	0	+	+	+	+	+	+	+	?	?	+	65
Muscular pains	?	+	+	+	+	+	+	+	+	?	?	+	74
Prostration	+	+	+	+	+	+	+	+	+	+	+	+	100
Conjunctivitis	0	0	0	+	0	+	0	0	0	0	0	+	25
Injection of pharynx	0	0	0	0	+	+	+	0	0	0	0	0	25
Herpes	0	0	0	0	+	0	0	+	0	0	0	+	25
Vomiting	+	+	0	+	+	+	0	+	0	+	0	+	65
Hiccup	0	+	0	0	+	0	0	0	0	0	0	+	25
Icterus	+	+	+	+	+	+	+	+	+	+	+	+	100
Cough	0	0	0	0	+	+	0	0	0	+	0	0	25
Hemorrhagic tendencies	0	+	0	+	+	+	+	+	0	0	0	+	58
Enlarged liver	0	0	+	+	+	+	+	+	+	0	0	+	65
Palpable spleen	0	0	0	0	0	0	0	0	+	0	0	0	8
Adenopathy	0	0	0	0	0	0	+	0	0	0	0	0	8
Outcome	R	D	R	R	D	R	R	R	R	D	D	D	% dead 41

* In the table, 0 indicates absence of the symptom; +, its presence; R, recovery of the patient; D, death, and ?, no mention.

It is of interest to note in this connection that Edelman [66] recorded a positive Wassermann reaction among his cases of rat-bite fever, a spirochetal diseases due to Spirochaeta morsus-muris.

66. Edelman, S. D., and Haber, G. B.: J. Pediat. **5**:520, 1934.

The diagnosis was verified in all instances by examination of the blood by dark field, by inoculation of guinea-pigs or at necropsy. Forty-one per cent of the cases terminated fatally. This seems a high rate of mortality, but it is best explained on the supposition that the milder cases without jaundice (in which the mortality rate is low) were not diagnosed.

DIFFERENTIAL DIAGNOSIS

Two common types of jaundice exist in this country which are confused with each other and with Weil's disease. 1. Infectious jaundice, as Blumer,[67] Wadsworth [4] and other investigators have shown, occurs as a benign and highly contagious disease in this country. The etiology is unknown, and the study of hundreds of cases has not once shown it to be due to a spirochete. It is characterized by appearing in epidemic form, mostly in the fall and winter, and by attacking primarily children and adolescent boys and girls. It is rarely fatal and lacks the renal and hemorrhagic manifestations of Weil's disease. 2. Catarrhal jaundice clinically resembles the afebrile type of infectious jaundice and is looked on by some investigators as the sporadic form of this disease. However, Held and his associates [68] pointed out that it is essentially a degenerative involvement of the polygonal hepatic cells, which are rendered susceptible by loss of glycogen to the action of an as yet unknown toxic substance. Soffer [69] showed that one attack may leave permanent damage to the liver. It is rarely fatal; it is noncontagious and attacks primarily persons under the age of 40.

The possibility of acute yellow atrophy may be confusing but should be ruled out on the basis of the clinical course, shrinkage of the liver and the finding of leucine or tyrosine crystals in the urine.

When jaundice is absent (or before it appears) Weil's disease may simulate trichiniasis (muscular pain and ocular signs), nephritis (renal injury), dyscrasias of the blood (hemorrhagic tendencies), severe generalized infection (chill, fever, malaise and prostration), meningitis (headache and stiff neck) and gastro-enteritis or enteric fever (nausea, vomiting, abdominal pain or diarrhea).

Relation to Yellow Fever.—Weil's disease has been confused with yellow fever because of the isolation of Leptospira by Noguchi from the blood of patients with supposed yellow fever and because of the extraordinary similarity of the clinical syndromes. It has been conclusively shown, however, that Noguchi's organism and the leptospira of Weil's

67. Blumer, G.: J. A. M. A. **81**:353, 1923.

68. Held, I. W.; Goldbloom, A. A., and Kramer, M. L.: Internat Clin. **4**:197, 1931.

69. Soffer, L. J., and Paulson, M.: Arch. Int. Med. **53**:809, 1934.

disease are serologically identical.[70] The blood of patients with yellow fever is pathogenic primarily for Macacus rhesus rather than for the guinea-pig. Yellow fever convalescent serum protects the monkey from the virus of yellow fever but has no effect on L. icterohaemorrhagicae, whereas convalescent or immune horse serum of Weil's disease protects against the spirochetes (agglutinates and dissolves them) and fails to protect against the virus of yellow fever. The obvious and generally recognized explanation, therefore, is that the spirochetes in the material received by Noguchi were obtained from patients with Weil's disease contracted by accident during the outbreak of yellow fever. Two such instances have since occurred in the epidemic in 1928 in Rio de Janeiro.[71] No spirochete has ever been recovered from patients with West African yellow fever, and the disease has finally taken its place among those attributed to a filtrable virus.

Schüffner convincingly showed that the decision of "Weil or no Weil" rests with the bacteriologist and the pathologist. The bizarre symptomatology and marked variation in severity of Weil's disease have caused considerable confusion and have made its recognition on purely clinical grounds often impossible. Hence, in all suspected cases the diagnosis should be established or rejected by means of the laboratory procedures previously described.

TREATMENT

As long as the disease is rare and sporadic in this country treatment will be symptomatic. However, it should be recognized that a highly potent serum has been developed in Europe and Japan. With serum treatment, as noted by Inada, there are reduction in the mortality rate, distinct amelioration of the symptoms and shortening of the course of the disease. The polyvalent serum of Baermann and Smith produced a surprising effect even in the severest, most critical cases or even when given relatively late. The patients often felt better in a few hours.[34] Instances of surprisingly successful results from serum treatment in Weil's disease have repeatedly been observed in the Netherlands.[72]

The serum should be given preferably during the first few days, intravenously and in large doses. Inada gave 60 cc. in twenty-four hours, 40 cc. the first day and 20 cc. the next or 20 cc. on each of three consecutive days.[54] Baermann gave from 60 to 90 cc. the first day and then from 30 to 40 cc. every other day until a total of 200 cc. was

70. Theiler, M., and Sellards, A. W.: Am. J. Trop. Med. **6**:388, 1926; **7**:369, 1927.

71. Müller, H. R., and Tilden, E.: J. A. M. A. **94**:856, 1930.

72. Schüffner, W., and Mochtar, A.: Zentralbl. f. Bakt. (Abt. 1) **101**:405, 1927.

used in very severe cases. No unfavorable reaction of consequence was ever noted.

Serum is positively indicated, if available, for patients with severe symptoms whose disease has been diagnosed and for laboratory workers with a clear history of accidental exposure several days or more before the onset of symptoms.[73] "In any event," wrote Uhlenhuth, "according to all past experience, experimental and clinical, the early use of large doses of specific serum must be urgently recommended for the treatment of Weil's disease."

Because of the heterogeneity of the various strains of Leptospira (as indicated by minor differences in titer required for agglutination), the serum should be widely polyvalent. Noguchi used nine strains from North and South America, Japan, England and France; yet his serum failed to affect certain other strains. Baermann used one hundred Sumatran strains and fifteen others, including strains from the Netherlands, Germany and France. Organisms of all degrees of virulence were represented.[34]

Standardization of the serum must for the present be based on the agglutination or lysis titer of existing fresh virulent strains. Baermann's noteworthy results were obtained with serums the titer of which ranged from 1 : 1,000 to 1 : 10,000.

Rapid production of serum for use in emergency (as in an epidemic) was described by Uhlenhuth.[15] He found that adult rabbits given a single large dose of organisms survived the period of jaundice and produced antibodies of considerable value much earlier than they were produced in the horse or sheep. Properly preserved serum should be efficacious after several years of storage. That of Griffith, which contained 0.4 per cent phenol and was kept in a cool dark cupboard, maintained its original potency for seven years.[74]

Equally good results have followed the use of convalescent serum obtained from patients recovered from the disease in whom high agglutinating and lytic titer are known to have developed. It would be an excellent plan if in the future convalescent serum were collected from each person recovering from Weil's disease in this country.

Arsphenamine and other arsenical preparations have been shown by numerous clinicians to be ineffective as a spirocheticidal agent in Weil's

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73. As far as we have been able to determine, serum is not at present available in this country. Manufacture of Noguchi's serum was discontinued after it was found nonspecific for yellow fever. According to Fairley,[11] serum is obtainable from Burroughs, Wellcome & Co., London, and we suggest that it might be procurable through their New York agency.

74. Griffith, A.: J. Hyg. **18**:59, 1919.

disease. They may further damage the liver. A soluble preparation of bismuth (bismuto-chiniofom) has been found remarkably effective in guinea-pigs.[75] Although no reports of its use in man are available in the literature, it seems worthy of trial in severe cases if serum is not available. The symptomatic treatment is essentially similar to that for hepatitis, nephritis or severe generalized infection.

GENERAL COMMENT

In a recent editorial in *The Journal of the American Medical Association* it was stated that "Weil's disease seems to be one of those conditions fated to play an ever larger part in medicine and public health." [76] Evidence has been brought forward in this paper which we believe amply substantiates this prophecy.

Certainly, the same potential factors for the spread of the disease exist in this country as in other countries, and it has been shown that: (1) the Weil strain of Leptospira is the same the world over; (2) at least 10 per cent of wild rats in the United States harbor and excrete virulent organisms; (3) occupational exposure exists (most of the 12 reported cases occurred in persons working at certain trades); (4) spirochetes have been found to thrive in water in many places in this country, and (5) sporadic cases of Weil's disease (clinically and pathologically identical with the classic European variety) continue to be reported at intervals.

That lack of recognition of the disease accounts for the report of so few cases in the United States has been commented on. A condition comparable to that obtaining in this country existed in Scotland, France, the Netherlands and other countries about ten years ago, when Weil's disease was rarely reported to the health authorities. During recent years (owing almost entirely to a better understanding of this disease entity), it has become commonly recognized. Physicians in the aforementioned countries consider Weil's disease frequently in their differential diagnoses. It has become as common there to send blood to the health laboratories for agglutination with Leptospira as it is in this country to send blood to a state laboratory for a Widal test. A typical example is the Pasteur Institute of Paris, where during 1933, 1,232 specimens of blood from persons suspected of having Weil's disease were examined, and agglutination with Leptospira [77] was obtained in 23.1 per cent.

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75. Uhlenhuth, P., and Seiffert, A.: Zentralbl. f. Bakt. (Abt. 1) **114**:241, 1929.

76. Editorial, J. A. M. A. **103**:493, 1934.

77. Erber, B.: J. Bull. Office internat. d'hyg. pub. **24**:1749, 1934; abstr., Brit. M. J. **2**:1155, 1934.

The true incidence and future course of Weil's disease in America will be better determined if the following conditions are fulfilled:

1. The disease must be suspected more often and the variation in symptomatology appreciated.

2. All suspected cases should have laboratory confirmation.

3. Laboratories (both public health and hospital) should be prepared to make the necessary diagnostic tests.

4. Serum and prophylactic measures should be utilized when indicated.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—At the University of Michigan Malcolm H. Soule, professor of bacteriology, has been made director of the hygienic laboratory and chairman of the department of bacteriology in succession to F. G. Novy, who has retired.

Ernest E. Tyzzer, professor of bacteriology in the Harvard University Medical School, has received the honorary degree of doctor of science from Brown University.

C. R. Bardeen, professor of anatomy and dean in the medical school of the University of Wisconsin, has died at the age of 64.

Walter J. Nungester, assistant professor of bacteriology in the medical school of Northwestern University, has been appointed associate professor of bacteriology in the University of Michigan.

Albert C. Broders, of the Mayo Clinic, has been appointed professor of surgical pathology and director of cancer research at the Medical College of Virginia, Richmond.

Broman C. Crowell, director of clinical research in the American College of Surgeons, Chicago, has been elected president of the Gorgas Memorial Institute.

A. R. Dochez has been elected a member of the board of scientific directors of the Rockefeller Institute for Medical Research.

John Weinzerl, professor of bacteriology in the University of Washington since 1912, has died at the age of 65.

The Poor Richard Club of Philadelphia has awarded its medal of achievement to John A. Kolmer in recognition of his work on prevention of epidemic poliomyelitis.

N. Paul Hudson, professor of pathology in the University of Chicago, has accepted an appointment as professor and chairman of the department of bacteriology in Ohio State University, in place of Charles B. Morrey, who has resigned.

Floyd S. Markham, J. M. Birkeland and O. C. Woolpert have been appointed assistant professors of bacteriology in Ohio State University.

A bronze plaque of Alfred S. Warthin, who died in 1931, has been presented to the University of Michigan by persons who worked with him in the department of pathology under his directorship. Dr. Warthin was connected with the university from his graduation there in 1891; since 1903 and until his death he was professor of pathology and director of the pathologic laboratory.

Ralph S. Muckenfuss, assistant professor in Washington University, St. Louis, has been appointed temporary assistant director of the bureau of laboratories of the department of health of New York City.

Society News.—At the annual meeting of the American Society of Clinical Pathologists Roy R. Kracke was chosen president-elect; R. A. Kilduffe, vice-president, and A. S. Giordano, secretary-treasurer.

The forty-sixth annual meeting of the Association of American Medical Colleges will be held at Toronto, Canada, on Oct. 28, 29 and 30, 1935.

Corrected Notice.—The Second International Congress for Microbiology will be held in London, July 25 to Aug. 1, 1936.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

PURE CULTURES OF FIBROBLASTS FROM SINGLE MONONUCLEAR CELLS. J. K. MOEN, J. Exper. Med. **61**:247, 1935.

Most guinea-pig mononuclear exudative cells in tissue culture become typical migrating macrophages, but a small proportion take on fibroblastic characteristics and produce pure colonies of fibroblasts. These fibroblasts maintain their morphologic characteristics through repeated subcultures. It is suggested that the development of individual mononuclear cells in tissue culture subsequent to isolation is conditioned at the time of explantation. Apposition to other cells is not necessary for the initiation of mitotic cellular division. There is a definite optimal relationship between the bulk of the medium, the number of explanted cells and the extent of proliferation. The presence of other cells in the vicinity enhances cellular division. Mitosis in the isolated explanted cell is preceded by a latent period. The rate of division varies in different colonies of fibroblasts. Admixed erythrocytes in the mononuclear suspension definitely inhibit proliferation of fibroblasts in tissue culture. The inhibiting factor in disintegrating erythrocytes is apparently in the stroma.

FROM THE AUTHOR'S SUMMARY.

BLOOD PLASMA PROTEIN REGENERATION CONTROLLED BY DIET. W. T. POMMERENKE ET AL., J. Exper. Med. **61**:261 and 283, 1935.

1. *Standardization of Food Proteins, Fasting and Iron Feeding.*—When blood plasma proteins in dogs have been depleted by bleeding, with return of washed red cells (plasmapheresis), it is possible to bring the animals to a steady state of low plasma protein and uniform production of plasma proteins on a basal diet. Such dogs are excellent test subjects by which to measure the potency of various dietetic factors for the regeneration of plasma proteins. To regenerate plasma proteins in any significant amount the depleted dog requires food proteins. Some proteins are very potent for the production of new plasma proteins, and others are utilized poorly. Beef serum is potent; the proteins in 2.6 Gm. of serum will produce 1 Gm. of new plasma proteins in the depleted dog—a potency ratio of 2.6. Kidney protein stands at the bottom of the list; the dog needs 21 Gm. of kidney protein to regenerate 1 Gm. of plasma protein—a potency ratio of 21. Some grain proteins approximate beef serum in potency and may show potency ratios of from 2.7 to 4.6. Some of these grain proteins appear to favor the production of globulin more than that of albumin in the plasma. Skeletal muscle, gizzard (smooth muscle), lactalbumin and egg-white fall into a favorable group with a potency ratio of from 5.3 to 6. Whole liver, liver fractions, casein and beef heart are a little less potent, presenting potency ratios of from 6.5 to 8. Many of these food substances favor the production of albumin more than that of globulin. Pancreas and salmon muscle show less favorable potency ratios of 19 and 15, respectively. Fasting periods indicate that the depleted dogs can produce little if any new plasma protein. Iron feeding in some unexplained manner influences body metabolism so that an excess of plasma protein is produced. These observations have a bearing on clinical conditions associated with hypoproteinemia and give suggestions for dietary aid or control in some of these abnormal states. The make-up of the diet is obviously of great interest, and it is possible that combinations of proteins may be more potent than a single protein or that food potency ratios may differ in health and disease.

2. *Dog Plasma Protein, Horse Plasma and Dog Hemoglobin.*—Foreign plasma protein (horse) introduced parenterally into the dog deprived of dietary protein is not utilized in the body economy. Its fate appears to be disintegration and elimination as excess urinary nitrogen. This is totally different from the fate of dog plasma protein under similar conditions. Dog hemoglobin given parenterally to the dog deprived of protein is not utilized as is dog plasma protein to keep the animal in nitrogen equilibrium; the globin is largely broken down and discarded as excess urinary nitrogen. A small part of the injected hemoglobin is probably utilized to maintain the red cell concentration in the blood at high levels. Dog plasma given parenterally in a dog deprived of dietary protein will maintain the dog in nitrogen equilibrium, and there is no surplus nitrogen elimination in the after-periods. It is apparent that the introduced plasma protein is utilized efficiently in body metabolism to replace or repair tissue protein. It is suggested that although this is an emergency reaction the same reaction may go on in normal internal metabolism. The observation that foreign plasma and dog hemoglobin cannot be utilized when given parenterally strengthens the argument for a normal contribution from plasma proteins to body proteins.

FROM THE AUTHORS' SUMMARIES.

RENAL DAMAGE FROM A DIET CONTAINING EXCESS INORGANIC PHOSPHATE. E. M. MCKAY and J. OLIVER, J. Exper. Med. **61**:319, 1935.

The addition of an excess of inorganic phosphate in the form of orthophosphoric acid, acid, basic or neutral sodium or potassium phosphate to the diet of albino rats results in the development of an interesting and permanent renal lesion. The lesion is characterized by necrosis of the cells of the convoluted tubules commencing at the terminal end, followed by atypical regeneration of epithelium and calcification of the necrotic débris that fills the tubules. The entire outer stripe of the outer zone of the medulla is transformed into a zone of distorted structures, and there is an increase in the interstitial connective tissue. The adjoining cortex is also involved with cystic dilatation of tubules and collapse. Such areas may reach the free surface of the organ and produce retracted scars. In the gross the kidneys are enlarged and firm on section, with a pebbled surface produced by numerous scars. The maximum changes in the kidney structure are reached after about fifteen days, but necrosis of the cells of the convoluted tubules is evident after a single day of phosphate feeding. The renal structure is not restored to its normal form when the excess of phosphate is removed from the diet.

FROM THE AUTHORS' SUMMARY.

MOTTLED ENAMEL IN CATTLE. H. T. DEAN, Pub. Health Rep. **50**:206, 1935.

An additional area, west Texas, showing mottled enamel in cattle is reported. The economic consequence of widespread fluorosis in stock may be a problem of some significance in animal husbandry.

FROM THE AUTHOR'S SUMMARY.

THE ACTION OF ACETONE AND OF THE KETONE BODIES PRESENT IN DIABETIC BLOOD UPON THE HEART. M. M. BAGOURY, Brit. J. Exper. Path. **16**:25, 1935.

Different concentrations of the ketone bodies present in diabetes were compared as to their action on the heart muscle and the minimal effective doses determined. Acetone and aceto-acetic acid produce a weakening of the heart, as evidenced by the cardiac dilatation and the rise of the venous and pulmonary pressures. The toxicity of aceto-acetic acid is about from fifteen to twenty times greater than that of acetone. The minimal effective concentration of aceto-acetic acid is from 2 to 3 mg. and that of acetone from 25 to 40 mg. per hundred cubic centimeters of blood. The weakening effect of acetone remains unchanged so long as the acetone remains in the circulation. On replacing the blood by acetone-free blood the heart recovers completely. The cardiac dilatation produced by

small doses of aceto-acetic acid tends to disappear spontaneously. Beta-oxybutyric acid has no specific action on the heart up to concentrations of 1 per cent. Its effects are entirely accounted for by the changes in the acid-base equilibrium which the acid produces in the blood. The three ketone bodies produce an increase in the coronary blood flow. This effect is, however, negligible unless the concentration of these substances in the blood is very high. Administration of dextrose and of insulin does not modify the action of these three substances on the heart muscle.

FROM THE AUTHOR'S SUMMARY.

Pathologic Anatomy

HISTOLOGICAL EFFECTS OF POTASSIUM IODIDE AND THYROID SUBSTANCE ON THE THYROID GLAND OF THE GUINEA PIG IN SCURVY. W. F. ABERCROMBIE, Am. J. Path. **11**:469, 1935.

In scurvy the thyroid gland presents irregular follicles with higher epithelium, a reduced amount of nonuniformly stained and extensively vacuolated colloid and an increase in the interfollicular cells. These changes are more marked in chronic scurvy of long duration than in acute scurvy. Potassium iodide causes a decrease in the number of vacuoles and an increase in the amount of colloid, accompanied by a flattening of the epithelium and a decrease of the interfollicular cells. Thyroid substance produces similar results except that the epithelium is not flattened but is returned to the normal medium height. Potassium iodide and thyroid substance, in the doses administered, do not tend to prolong the life of the scorbutic animal. Thus, it appears that vitamin C is not concerned with iodine metabolism.

FROM THE AUTHOR'S SUMMARY.

GLOMERULAR CHANGES IN ARTERIOSCLEROTIC CONTRACTION OF THE KIDNEY. P. KIMMELSTIEL, Am. J. Path. **11**:483, 1935.

In arteriosclerotic kidneys the following degenerative changes can be recognized in the glomeruli:

A. Primary broadening and hyalinization of the intercapillary axial connective tissue. This very frequent change is interpreted as a phenomenon of the aging of the glomerulus and may lead to damage of the glomerular capillaries.

B. Thickening of the basement membrane, which is always secondary. It may be due to two different causes:

1. Ischemic atrophy of the glomerulus, which may result from: (*a*) direct encroachment of hyalinization of the vas afferens on the glomerulus leading to collapse and degeneration of all the glomerular elements, and (*b*) narrowing of the larger vessels producing slow circulatory atrophy of the tubules and glomeruli. This change, the most common in all forms of arteriosclerotic kidney, is characterized by thickening of the capsule and basement membrane, frequently extending from the former to the latter. This thickening of the capsule is closely associated with atrophy of the tubular epithelium.

2. Ascending atrophy. This is caused by obstruction of the corresponding tubules and is characterized by thickening of the capillary basement membrane without thickening of the capsule and is associated with dilatation of the capsular space. This form usually is not observed in pyelogenic ascending contraction. The latter is interpreted mainly as an ischemic process, thereby explaining the fact that in this condition one so frequently encounters a high degree of capsular thickening (vide 1b).

Tubular atrophy in arteriosclerotic kidneys is chiefly circulatory and essentially depends on changes in medium-sized and larger vessels.

FROM THE AUTHOR'S CONCLUSIONS.

BLOOD-FILLED CYSTS ON THE CARDIAC VALVES IN INFANCY. S. D. MILLS, J. Pediat. **6**:51, 1935.

Small blood-filled cysts occur not infrequently on the leaflets of the cardiac valves in the neonatal period. The best explanation for their origin is that they are cystic dilatations at the ends of endothelium-lined canals in the substance of the valves.

FROM THE AUTHOR'S SUMMARY.

THE HISTOLOGY OF THE TUBERCULOUS CAVITY WALL. S. R. GLOYNE, Tubercle **16**:161, 1935.

In the cavity of the acute bronchopneumonic tubercle the connective tissue is minimal, and the parenchyma of the lung still forms the greater part of the wall. In the old cavity with a thick wall the latter consists entirely of dense connective tissue which has obliterated all other structures with the exception of bronchi which contain portions of cartilage enabling them to resist compression. Between these two extremes are those which may be designated the cavities of the intermediate stage. No single description will fit the walls of all; their structure is varied. There is always more connective tissue in the wall than examination with the naked eye suggests. Setting aside the somewhat rare small cavity of the acute bronchopneumonic tubercle, one may safely infer that by the time a cavity is recognizable in the roentgenogram connective tissue is already formed in its walls.

H. J. CORPER.

THE LESIONS OF THE CHRONIC FORM OF PERIARTERITIS NODOSA. MACAIGNE and P. NICAUD, Ann. d'anat. path. **11**:235, 1934.

In an adult with recurrent attacks of asthenia, polyneuritis, polymyositis and multiple nodules of the skin of about ten years' duration biopsies of the nodules revealed arterial and arteriolar changes considered characteristic of periarteritis nodosa: endothelial proliferation with thickening of the intima and narrowing of the lumen; doubling of the elastica interna; separation of the muscle fibers of the media and infiltration of the adventitia by granulation tissue and fibrosis. Foci of necrosis and occasionally suppurative changes were present. Inoculation of material from the lesions into two guinea-pigs and a monkey yielded no results.

PERRY J. MELNICK.

PRIMARY POLYMYOSITIS. G. MARINESCO, S. DRAGANESCO and E. FAÇON, Ann. d'anat. path. **11**:537, 1934.

The authors report a case of primary hemorrhagic nonsuppurative polymyositis (Frenkel-Wetzoldt). Microscopically the muscles showed extensive degenerative changes but also marked inflammatory changes and hemorrhages. Various fixatives and stains were used so that artefacts were not a confusing element. Different degrees of degenerative changes progressing to coagulation and necrosis were seen. The inflammatory process had the character of an interstitial subacute infiltration of round cells, capillaries and connective tissue. The authors consider the condition to be inflammatory, and differentiate it from muscular dystrophies, myasthenia gravis, Landry's syndrome, rheumatism, trichinosis, neuromyositis and dermatomyositis.

PERRY J. MELNICK.

HISTOLOGIC STRUCTURE OF THE REMAINING PORTION OF THE THYROID AFTER CURE OF EXOPHTHALMIC GOITER BY SUBTOTAL THYROIDECTOMY. G. ROUSSY, R. HUGUENIN and H. WELTI, Ann. d'anat. path. **11**:555, 1934.

In two cases the authors made biopsies of the portion of thyroid gland remaining after subtotal thyroidectomy for thyrotoxicosis, in one case two years, in another case five years, after the operation. The specimens were obtained during cosmetic

repair of the scars. In both cases the histologic picture was identical with that of the goiters previously removed. In both, although the organ was clinically cured, with normal physiology reestablished, the thyroid tissue was found to be as hyperplastic as the original goiter.

PERRY J. MELNICK.

STUDIES OF THE INTERMEDIATE LOBE OF THE HYPOPHYSIS. G. ROUSSY and M. MOSINGER, Ann. d'anat. path. **11**:655, 1934.

The authors made a histologic study of the hypophyseal fissure, the diverticula of the fissure, the sero-albuminous glands of the intermediate lobe and the cellular infiltrations of the posterior lobe. They studied 112 normal hypophyses from adults of various periods of life ranging to advanced age, 6 from infants and children, and 1 from an 8 month fetus. In the fetus and the infant the hypophyseal fissure is intact. In both the anterior and posterior walls the lining epithelium is the same. Therefore the anterior wall properly belongs to the intermediate lobe. Besides flat, cuboidal and cylindric cells a fourth type of cell is found in this epithelial layer, namely, a basophilic branched cell apparently capable of migration. From the fissure of the hypophysis diverticula branch off in all directions, including diverticula into the posterior lobe. These are different from the sero-albuminous glands of Erdheim in the intermediate lobe, which are lined by a different type of secreting epithelium. Cellular infiltrations into the posterior lobe consist, in infants, of basophilic cells from the intermediate lobe, but in adults they may also consist of eosinophilic cells from the anterior lobe since the fissure is obscured and there is a more direct continuity with the anterior lobe. These anatomic structures point the way to interesting conclusions regarding the various physiologic and pathologic states of the hypophysis.

PERRY J. MELNICK.

CHANGES IN THE ENDOCRINE GLANDS IN DIABETES MELLITUS. M. LABBÉ and M. PETRESCO, Ann. d'anat. path. **11**:761, 1934.

The hypophysis, thyroid and adrenals were studied histologically in twenty-eight cases of diabetes mellitus, in some of which the condition was associated with pigmentation. The following changes were found: The hypophysis usually showed a diminution in size and weight associated with a decrease in number and with degenerative changes of the eosinophilic cells of the anterior lobe. In the thyroid more or less epithelial hyperplasia was detected. In most cases the adrenals were diminished in size and showed an associated decrease in lipoid content and regressive changes in the cells of the cortex. In four cases of severe diabetes there was sclerosis of the adrenal medulla. The authors conclude that the lesions of the islands of Langerhans are fundamental in diabetes. They do not admit that the other endocrine glands play a rôle in the regulation of glycogen. There exist changes in the other endocrine glands of secondary importance, which however explain certain syndromes sometimes associated with diabetes, such as thyrotoxicosis, genital dystrophy, hypertension, etc.

PERRY J. MELNICK.

THE RETICULAR REACTIONS AND FUNCTIONS OF THE SPLENIC LYMPH FOLLICLES. J. WÄTJEN, Centralbl. f. allg. Path. u. path. Anat. **62**:1, 1935.

Retrogressive and progressive changes have been noted in the splenic lymph follicles after poisoning with certain karyoklastic substances. The regressive changes in the lymphatic cells were proportionate to the progressive changes in the reticulum. Questions then arose as to whether the proliferative reaction followed lymphatic damage or whether it was a primary reaction and whether this was an antitoxic reaction. Haranghy answered these questions positively from observations in diphtheria and in ricin poisoning. Wätjen is concerned with corroboration of this work and in many respects is in accord with Haranghy. Changes in the lymph follicles are variable because the germinal centers have an upward and a downward phase and are more easily affected by poisons in the full development or early regression stages. Changes after ricin poisoning are apparent within

eighteen hours, and iron pigment is encountered at this period and thereafter in the reticulum cells of the follicles. This feature is important because it demonstrates activity in support of the reticulum cells of the pulp which usually exercise such a function. It is seen only after rarefaction and activation of the follicle have occurred. In protracted ricin poisoning a perinodular leukocytic wall is noted, and this speaks for the nodules acting as poison depots and exerting a chemotactic effect. The changes noted in diphtheria in human beings can be compared only tentatively with those noted in experimental ricin poisoning in guinea-pigs. In those dying of toxic diphtheria early, nuclear disintegration is found in the follicles. Those who withstand the disease longer have a stimulation of the reticulum even to the formation of epithelioid centers. These changes are indicative of the action of a weak poison.

GEORGE RUKSTINAT.

DILATATION OF THE ESOPHAGUS. H. ARNOLD, Centralbl. f. allg. Path. u. path. Anat. **62**:49, 1935.

The condition forming the basis for this discussion was encountered post mortem in a woman, aged 49 years, who died of cardiac decompensation and mitral stenosis. Symptoms referable to the esophagus dated from childhood, when a feeling of fulness after eating and vomiting of food taken the previous day were noted. At the ages of 15 and 20 years sounds had been passed in the esophagus, and a condition of stenosis diagnosed. The stenosis involved the lower half of the esophagus, while the upper half was dilated. At necropsy the esophagus was 32 cm. long, and its circumference varied between 8 and 10 cm. The upper third was altered by a granular esophagitis. There was an ulcer at the pylorus. Arnold believes that the dilatation described was present long before the age of 15 years and was inaugurated by cardiospasm. He emphasizes the fact that functional difficulties such as cardiospasm or achalasia are manifested earlier in life and more strongly than are such organic alterations as carcinoma of the cardia. The same observation holds true elsewhere in the gastro-intestinal tract as in pylorospasm or Hirschsprung's disease. During adolescence and early adult life compensation in the form of muscular hypertrophy was ample to overcome the stenosis. The waning response with approaching old age was further hindered by the marked mitral stenosis and periodic cardiac decompensation.

GEORGE RUKSTINAT.

A BONE MARROW NODULE IN THE PARARECTAL FAT. H. KUDLICH, Centralbl. f. allg. Path. u. path. Anat. **62**:83, 1935.

In a woman, aged 49 years, who died from pneumonia a mass the size of a walnut was found in the fat between the rectum and the sacrum. On microscopic examination this proved to be composed of typical bone marrow. Kudlich briefly reviews the literature on foci of bone marrow in such locations as the adrenal glands or adrenal rests. He is certain that the sympathicoblasts or round cell collections found in some of the recorded marrow masses of the adrenal glands could play no part in the production of the pararectal growth which he encountered. He points out the observations of Petri and Gruber that the fat of human embryos in its development goes through a preliminary stage that resembles bone marrow. The growth in the present case might be regarded then as a local lack of development. The claims of Herzenberg and Patrassi that such marrow tissue might have a postfetal development from the endothelial cells of blood vessels are also cited. Either of the latter views could account for the development of bone marrow in any part of the body.

GEORGE RUKSTINAT.

TOTAL CAVERNOUS TUBERCULOSIS OF THE LEFT LUNG. L. ELLIOTT SILTZBACH, Virchows Arch. f. path. Anat. **292**:652, 1934.

Under the designation "left-sided total cavernous lung" Siltzbach presents from Erdheim's institute the roentgenographic and the gross and microscopic observations in nine cases of a form of pulmonary tuberculosis that has received scant attention.

All the patients were women whose ages varied from 22 to 39 years; one was 54 years old. The clinical duration of the illness in eight patients ranged from five to twenty years; in one patient it was one year. The left lung was involved in each instance. Of nine examples of a similar condition collected from the literature, two dating back to 1803 and 1847, five occurred in women. In eight of these cases the left lung was involved. In Siltzbach's cases the left lung had been transformed into one or two large cavernous spaces bounded by dense fibrous tissue derived from the adherent visceral pleura. Remnants of parenchyma could be detected in the lower but not in the upper lobe. In the fibrous walls of the cavities there were numerous focal aggregations of lymphocytes and a few miliary tubercles. There were also many encapsulated caseous areas. The lining of the cavities was formed by a zone of nonspecific chronic inflammatory tissue with leukocytic infiltration. The fibrous pleural adhesions were rich in elastic tissue. The hilar nodes contained tubercles. The right lung revealed older encapsulated caseous foci and areas of more recent tuberculosis. In a brief supplemental note two further cases are described, which also occurred in women and involved the left lung.

O. T. SCHULTZ.

WEIGHT OF THE THYMUS IN NEW-BORN INFANTS. P. R. RUSSKOFF, Virchows Arch. f. path. Anat. **293**:113, 1934.

The weight of the thymus, absolute and relative to body weight, was determined in 841 infants who came to necropsy in the pathologic institute of the University of Bern, Switzerland, during the years 1908 to 1932 inclusive. Premature and full-term infants who died within the first month of life were included, as were also stillborn infants. The material was divided into five groups according to body weights. In general, the weight of the thymus was slightly higher in male infants than in female infants of the same group. In a group of infants who were at least 50 cm. long and who lived not longer than twenty-four hours, the average weight of the thymus was 11.8 Gm. in males, and 12.5 Gm. in females, and in the two sexes it was 12.1 Gm. The relation of thymus weight to body weight was 1:271.8 (0.37 per cent). The thymus was heavier in anencephalic infants than in normal infants of the same weight. In the later months of fetal life the growth of the thymus parallels that of the body. The weights of the thymus and thyroid gland did not parallel each other. The average weight of the thymus in the goiter district of Bern did not exceed that of other regions.

O. T. SCHULTZ.

CARTILAGE RESTS IN THE TONGUE. C. GENTSCHEFF, Virchows Arch. f. path. Anat. **293**:129, 1934.

In carnivorous animals and in swine there occurs normally at the inferior margin of the anterior portion of the septum of the tongue a wormlike fibrous thickening that has been known as the "lyssa" or "fury worm"; its name indicates that superstition ascribed to it a rôle in rabies. This structure may contain small islands of cartilage. In 1895 Nusbaum and Markowski noted the occurrence of islands of cartilage in a similar situation in the tongues of 30 per cent of new-born infants and fetuses of the eighth and ninth months examined by them; according to these authors, the cartilage disappears in later life. The accidental discovery of islands of cartilage in the tongues of two adults led Gentscheff to make further investigation of the matter. Including the two cases first observed, cartilage was found in the tongue in nine of thirty-four subjects of necropsy; three times in infants from 1 to 16 days old, once in a child 4½ years old, and five times in adults aged from 57 to 68 years. The cartilaginous islands, which may be single or multiple, were always situated approximately 1 cm. from the tip of the tongue at a depth of from 1.5 to 4.5 mm. from the inferior surface. Gentscheff concludes that the cartilaginous islands are phylogenetic skeletal rests of the cartilaginous rod of the reptilian tongue and that they correspond to the lyssa of the tongue of carnivorous animals.

O. T. SCHULTZ.

THE AXIAL SKELETON IN ANENCEPHALY AND CRANIORACHISCHISIS. B. DEPPE, Virchows Arch. f. path. Anat. **293**:153, 1934.

Study of a series of fetuses presenting varying grades of anencephaly and craniorachischisis led Deppe to conclude that maldevelopment of the central nervous system is the primary anatomic factor, to which the failure of union of the bones of the cranium and vertebral column is secondary. Ribs and vertebral bodies are formed in normal numbers but are often fused. The characteristic distortions of the spinal column are the result of mechanical factors, such as pressure and muscular traction, to which the open vertebral column is less resistant than the closed one.

O. T. SCHULTZ.

CHANGES IN THE CENTRAL NERVOUS SYSTEM DUE TO AN ELECTRIC CURRENT. S. JELLINEK and E. POLLAK, Virchows Arch. f. path. Anat. **293**:165, 1934.

Death usually results so quickly after electric shock that the central nervous system reveals only slight changes. If necropsy is delayed, it may not be possible to decide to what extent the changes noted are postmortem in character. The brains of two persons who died twenty-four hours and six days, respectively, after contact with an electric current were examined microscopically. In the second case the observations were complicated by the fact that death was due to tetanus. In both cases the most striking features were engorgement of the venules and arterioles and microscopic hemorrhages. The latter were most numerous in the tissue about the ventricular system, especially about the third and fourth ventricles and the aqueduct. In the first case there was also calcium incrustation of the small vessels and capillaries of the globus pallidus as well as deposition of calcium about the vessels in the form of droplets. The localization of this process is ascribed to the course and structure of the vessels of the globus, to which the changes noted after carbon monoxide poisoning are also ascribed. The authors postulate damage to endothelium and metabolic changes in it and in the immediately surrounding tissue.

O. T. SCHULTZ.

ARTEFACTS IN THE CENTRAL NERVOUS SYSTEM IN RELATION TO CHANGES ASCRIBED TO AN ELECTRIC CURRENT. F. BOEMKE, Virchows Arch. f. path. Anat. **293**:180, 1934.

Schridde has repeatedly maintained that in his large material he has never seen hemorrhages in the brain as the result of the passage of an electric current. Larger hemorrhages he ascribes to the fall which the electrically shocked person usually sustains. The minute capillary hemorrhages described by Jellinek, he maintains, are pure artefacts. In the work here reported, which was carried out in Schridde's institute, the brains of ten persons who had not died of electric shock were examined for changes previously ascribed to electric currents; these brains had been removed in the usual course of necropsies. In fifteen other necropsies the brains were somewhat more carelessly removed and were squeezed by the hands in removal. Not only in the second group of brains, but also in the first, the author claims to have observed all the alterations that others have ascribed to electric currents. Such changes are all artefacts due to the force used in removal of the brain. Minute areas in which the tissue does not take the myelin sheath stain well, described by Jellinek, are due to faulty staining technic, according to Boemke.

O. T. SCHULTZ.

Microbiology and Parasitology

APICAL LOCALIZATION OF PULMONARY TUBERCULOSIS. JEROME J. HURWICH and GEORGE MILLES, Am. Rev. Tuberc. **31**:151, 1935.

In monkeys following intravenous injection of tubercle bacilli many of the bacilli are picked up by the endothelial cells of the capillaries of the lungs. They are then ingested by macrophages and either enter the pulmonary veins to be distributed over the entire body or are excreted into the alveolar spaces. Apical

tuberculosis may be due to an excretion of tubercle bacilli from the blood stream into the alveolar spaces of the apexes, where aeration is deficient, the tubercle bacilli having reached the blood stream from the primary tuberculous complex.

H. J. Corper.

The Stability of the Colonial Morphology and Pathogenicity of BCG. Dorothy M. Behner, Am. Rev. Tuberc. **31**:174, 1935.

BCG (Bacillus Calmette-Guérin) did not acquire virulence in laboratory cultures on liquid mediums containing either normal human serum or homologous antiserum, or in the depths of nutrient broth, or by successive selection for propagation of smooth-appearing colonies on solid mediums, or by repeated passages through animals. The morphologic variation seen in the colony under different environmental conditions was not accompanied by enhanced pathogenicity. A virulent bovine strain was resistant to laboratory modification, but a slight attenuation in the virulence was produced together with a slight morphologic change in the colony, which acquired some characteristics usually associated with the rough variant. Avian strains were found to be labile, acquiring morphologic changes in colonial type in both S to R and R to S directions, and also some marked variations of each type, together with changes in pathogenicity under environmental conditions similar to those imposed on BCG strains.

H. J. Corper.

Lesions in Rabbits Following Inoculation with Bacillus Calmette-Guérin (B C G). W. H. Feldman, Am. Rev. Tuberc. **31**:323, 1935.

A strain of Bacillus Calmette-Guérin obtained from Calmette in 1930 and subsequently grown for twenty generations on an egg glycerin medium was transferred to glycerin peptone broth and with this culture six rabbits were inoculated intravenously and four guinea-pigs subcutaneously. One of the rabbits died ten days after inoculation and the other five were killed one hundred and seventy-four days after inoculation. Numerous and striking focal lesions morphologically like tubercles were found in the lungs of each of the five rabbits. Attempts to culture acid-fast bacteria from the lesions were futile, although bacteria of this character were readily demonstrable in appropriately stained sections of the lesions. Emulsions prepared from the involved tissues in each of the five rabbits failed to produce demonstrable lesions in other rabbits or guinea-pigs, and attempts to repeat the results in later experiments failed. The results indicate that Bacillus Calmette-Guérin in the lungs of rabbits may at times produce numerous and extensive tubercle-like lesions.

From the Author's Summary.

Trichinosis. W. W. Spink and D. L. Augustine, J. A. M. A. **104**:1801, 1935.

Thirty-five sporadic cases of trichinosis occurring in and around Boston during the past three years were analyzed. The most reliable diagnostic aid in these cases was the presence of eosinophilia. The skin test usually became positive about the seventeenth day of the infection and the precipitin test usually at the end of the fourth week. These tests were of especial diagnostic aid in the early stages of the disease, when they were first negative and later became positive. Mild sporadic and chronic cases of trichinosis were often detected only by these tests. Other laboratory procedures, such as searching for the parasite in the stools, blood and spinal fluid, were time-consuming and the larvae only rarely found.

From the Authors' Summary.

The Relation of Bacterium Granulosis to Trachoma. F. F. Tang and C. H. Chou, J. Infect. Dis. **56**:264, 1935.

Attempts to isolate Bact. granulosis from 179 cases of classic trachoma failed. The specimens used for cultivation consisted of lacrimal secretions, epithelial scrap-

ings, follicular contents and tarsectomized tissue. The mediums used were the semisolid "leptospira" medium of Noguchi and carbohydrate blood agar plates.

Attempts to induce trachoma in rhesus monkeys and in man by subconjunctival injection of cultures of Bact. granulosis failed.

A disease of the conjunctiva characterized by follicle formation but with no pannus formation or papillary hypertrophy was induced in two of nine monkeys inoculated with human trachomatous material. Bact. granulosis was not isolated at any time from either of these animals.

Antibodies against Bact. granulosis could not be demonstrated in the serums of twenty-six subjects suffering from acute or chronic trachoma.

From the Authors' Conclusions.

The Influence of the p_H on Dissociation of Bacillus Friedländer and Mycobacterium Tuberculosis. W. Steenken Jr., J. Infect. Dis. **56**:273, 1935.

B. Friedländer dissociates best in mediums of acid reaction. Myco-tuberculosis-humanis (H_{37}) becomes attenuated on acid synthetic mediums buffered with tenth-molar double potassium phosphate salts and on weakly buffered glycerol beef broth, and retains its pathogenicity on buffered alkaline synthetic medium prepared with double potassium phosphate salts. Acid glycerol potato bile medium favors attenuation of BCG. If BCG is grown on buffered synthetic medium over a period of a year or more it may regain its virulence. Stock medium weakly buffered above p_H 7 tends to become acid on standing in an incubator.

From the Author's Conclusions.

Experimental Brucelliasis in Dogs. W. H. Feldman, J. L. Bollman and C. Olson Jr., J. Infect. Dis. **56**:321, 1935.

Two strains of Brucella abortus obtained from a swine and a bovine source, respectively, were introduced into eleven adult mongrel dogs to determine (1) if they would induce a definite state of disease and (2) if there was any significant difference in their pathogenic behavior. Five of the dogs received the bacteria in suspension intravenously. Six were made to fast for twenty-four hours and then were fed the organisms mixed with raw meat.

Brucella agglutinins developed as early as the fourth day in the animals receiving the organisms intravenously. In this group titers of from 1:800 to 1:1,600 were not uncommon one week after the introduction of the organisms. Successive titers varied considerably in many cases. In the animals receiving the infective agent orally the agglutinative response was much slower and never so pronounced.

In a few cases it was possible to isolate Brucella abortus from the blood stream but only within two or three weeks after the introduction of the infective material. From two of the dogs it was possible to recover the organism in the urine.

Although most of the dogs lived for several months after receiving the bacteria clinical symptoms of disease and specific lesions of minor significance were observed in only one dog. From only two of the dogs were the organisms recovered after death; both had received the infecting inoculum intravenously, one dog thirty-nine days and the other one hundred and eighty-five days previously.

There was no discernible difference in the pathogenic propensities of the two varieties of Brucella abortus used.

While the dog is capable of producing Brucella agglutinins following experimental introduction of either the swine or the bovine variety of Br. abortus, a profound resistance to the organism exists which precludes, in most instances, the development of clinical symptoms and specific lesions.

From the Authors' Summary.

EXPERIMENTS WITH THE VIRUS OF INFECTIOUS ECTROMELIA. A. W. DOWNIE and C. A. MCGAUGHEY, J. Path. & Bact. **40**:297, 1935.

Growth of the virus of infectious ectromelia in tissue cultures could be inhibited for several days through the action of immune serum. Even when sufficient immune serum was present in the cultures to neutralize the virus (by in vivo tests) the virus was not killed. Multiplication of the virus in the presence of immune serum could always be demonstrated after from eight to twelve days' incubation. The state of the virus used as the inoculum was important in determining the effect of the immune serum. When a Berkefeld filtrate was used the inhibitory action of immune serum was readily demonstrated. With culture virus the tissue cells in the inoculum seemed to protect the virus from the immune serum and no inhibition of growth was apparent.

FROM THE AUTHORS' SUMMARY.

ISOLATION OF THE VIRUS OF PLEUROPNEUMONIA. F. F. TANG ET AL., J. Path. & Bact. **40**:391, 1935.

The virus was isolated by ordinary methods and cultivated on various mediums, particularly Bennett's broth (*J. Comp. Path. & Therap.* **45**:257, 1932). Five stages of development are described: elementary bodies (rings, granules and a few coccoid forms and rods); a stage in which filaments formed from elementary bodies; branching of filaments; formation of chains by the protoplasm in the filaments; disintegration of the chains into elementary bodies. No serologic differences could be made out in the different strains studied.

COMBINED CHEMOTHERAPY OF EXPERIMENTAL TRYPANOSOME INFECTIONS. C. H. BROWNING and R. GULBRANSEN, J. Path. & Bact. **40**:425, 1935.

In experimental infections with a strain of Trypanosoma brucei in mice it has been shown that combined therapy in which tryparsamide and a styryl compound are used in sequence produces a greater curative effect than follows the use of much larger doses of either substance alone. The evidence is strongly in favor of this result being more than a mere summation of effects. It is left undecided exactly how this "potentation" or "synergic" action is produced, but it should be noted that tryparsamide is quickly absorbed and excreted and its trypanocidal action is fairly rapid, whereas the styryl compound is slowly absorbed and acts gradually. Accordingly the advantage of combined treatment with the pair of drugs studied may be due to the prolonged influence of the styryl compound on parasites weakened by the arsenical drug, as well as to the fact that the substances differ widely in chemical constitution and so are likely to attack the parasites at different points (receptors).

FROM THE AUTHORS' SUMMARY.

TUBERCULOUS ARTERITIS. W. G. BARNARD, J. Path. & Bact. **40**:433, 1935.

A case of tuberculous inflammation of the internal carotid and coronary arteries in which there was no evidence of spread from a neighboring focus is described.

FROM THE AUTHOR'S SUMMARY.

STAPHYLOCOCCIC BACTERIOPHAGES. F. M. BURNET and D. LUSH, J. Path. & Bact. **40**:455, 1935.

A study has been made of the phages lysing Staphylococcus aureus and of the relation of these phages to those previously studied which lyse a white coccus "S. F." Differences among aureus strains in normal susceptibility to phage or resulting from acquired resistance are almost entirely of a nonspecific quantitative type. Normal aureus strains that are almost completely insusceptible to a strong phage adsorb it as readily as a susceptible strain. Strains with true induced resistance fail to adsorb phage. On general characters aureus phages can be divided into "strong" and "weak." All produce small sharp-edged plaques on agar. A

method of preparing high titer filtrates of weak phages by growth on cellophane agar at 22 C. is described. The aureus phages may be divided serologically into three distinct types. S. F. phages fall into four serologic types, one of which is common to the aureus phages. As with B. coli-dysenteriae phages all staphylococcic phages of a given serologic type react uniformly with regard to photodynamic inactivation by methylthionine chloride (methylene blue), power to grow on citrated mediums and inactivation by strong urea solution. Sharp distinctions may exist between different serologic types. There are clear resemblances between one serologic group of the strong aureus phages and the commonest type of small-plaque B. coli-B. dysenteriae phages (serologic group II of that series) which suggest a close biologic relationship between the two groups.

FROM THE AUTHORS' SUMMARY.

STUDIES OF TYPHUS. P. LÉPINE, Ann. Inst. Pasteur **51**:290, 1933.

The paper reports studies of "the existence of a murine typhus virus in the western Mediterranean Basin and its characteristics." A strain of virus producing scrotal reactions in guinea-pigs in a manner similar to that of Mexican typhus, and infecting guinea-pigs and monkeys, was found commonly in the brains of wild rats. The virus attacks man, resulting in a benign infection, very probably through fleas, which are regularly infected. The relationship to other similar exanthematous fevers is discussed.

M. S. MARSHALL.

Tumors

METASTASIS IN SQUAMOUS CARCINOMA. L. W. PRICE, Am. J. Cancer **22**:1, 1934.

The evidence available indicates that there is no correlation between the clinical condition of the patient and the development of distant metastases. There is no constant relationship between the site of the primary tumor and the site of the distant metastases. The commonest sites for metastases in this series were: lungs, thirteen cases; liver, seven cases; kidneys, five cases. Less frequently metastases were formed in other situations. From a wider consideration of the development of metastases from numerous primary tumors of various types the only consistent feature that emerges is that tumors arising in certain primary sites have a tendency to form metastases in certain tissues of predilection. There is a peculiar relationship between the site of the primary tumor and the site of the secondary deposits.

FROM THE AUTHOR'S CONCLUSIONS.

MELANOTIC NEOPLASMS OF THE SKIN. S. W. BECKER, Am. J. Cancer **22**:17, 1934.

Modern study of pigment, carried out mainly by means of the silver and dopa reactions, shows that at the junction of the epidermis and dermis there are specialized cells which are capable of forming pigment. The first sign of pigment activity in the embryo is a positive dopa reaction in a branched cell in this location. This is followed by the appearance of melanin granules in the branched cells and later in the palisade basal cells. These pigment-forming cells are called "melanoblasts" in contradistinction to phagocytic dermal cells which are called "chromatophores." An increase in the number of melanoblasts at the epidermo-dermal junction results in a smooth brown nevus. In elevated nevi masses of pale-staining cells are seen in the dermis which are similar in staining properties and pigment content to the epidermal melanoblasts and are thought to be derived from the same source. The source of melanoblasts is not definitely known, but more and more workers are accepting the nervous origin. If melanoblasts are located deep in the dermis a blue nevus or mongolian spot results. The distribution here is essentially the same as in the blue skin of the ape. Pigment activity due to irradiation by ultraviolet or alpha rays consists first of prominence and branching of melanoblasts, followed by hyperpigmentation of palisade basal cells. Pigment activity occurring spontaneously with no demonstrable cause results in the same

histologic picture, and the lesion is known as "lentigo," which has nothing to do with the common freckle known as "ephelis." If this stimulation of pigment activity increases to the point of melanoblastic proliferation, the lesion is known as "lentigo maligna" and is already malignant melanoma. Further activity results in melanoma of the fusiform cell type—the so-called melanosarcoma—or of the ovoid cell type known as "melanocarcinoma." The occurrence of both types of cell in the same primary or metastatic growth demonstrates the futility of trying to classify them as sarcoma or carcinoma, the best designation being "malignant melanoma." Melanoma arising from a pigmented nevus has its origin in the melanoblastic cells at the epidermodermal junction and not in deeply lying nevus cells as has been sometimes supposed. "Melanotic epithelium" and "pigmented epithelioma" are terms used to designate a benign epidermal neoplasm containing considerable melanin. These lesions are closely related to the so-called senile or seborrheic verruca and almost never undergo malignant degeneration. Study of cutaneous carcinomas showed that 33 per cent of the basal cell tumors, 14 per cent of the intermediate, 9 per cent of the mixed and 7 per cent of the squamous cell tumors contained melanin demonstrable by the silver technic. The pigment in these tumors is due to the presence of melanoblasts which cannot be distinguished from normal melanoblastic cells as regards type and arrangement of melanin granules. In rather unusual cases carcinoma of the breast which has invaded the skin is intimately associated with melanoblastic cells, which are also normal.

FROM THE AUTHOR'S SUMMARY.

EWING'S SARCOMA. C. L. CONNOR, Am. J. Cancer **22**:41, 1934.

From the three cases recorded here I believe one may say that an endothelioma of bone is much like a malignant endothelioma elsewhere, and that it does not respond readily to treatment with x-rays but disappears gradually and slowly as compared, for instance, with a metastatic lymphosarcoma or a neuroblastoma in bone, but at the same time much more rapidly and surely than an osteogenic sarcoma or the average metastatic carcinoma. Since my previous report I have discovered that metastatic lymphomatous tumors are the most difficult to differentiate from endothelial myelomas. The former may manifest themselves first as bone tumors, and it may not be until generalized lymphadenopathy in superficial areas occurs that the question of a lymphoma is raised. Perhaps there is more evidence that this tumor differs from a lymphosarcoma and other hematocytoblastic tumors in the fact that metastases to the lymph nodes were not present in any of these cases. Ewing's sarcomas constantly metastasize to the lungs, behaving in this respect like connective tissue sarcomas, in contrast to lymphosarcomas and myelomas. It may be found on further study that metastases to the lymph nodes are not common, and an opinion to the contrary, previously expressed, may have to be revised. Another revision can be made with the evidence at hand: The tumor cell of Ewing's sarcoma can become an osteoblast and form bone.

AUTHOR'S SUMMARY.

THE UTILIZATION OF SIMPLE DERIVATIVES OF GLUCOSE BY MOUSE SARCOMA. O. O. MEYER, C. MCTIERNAN and W. T. SALTER, Am. J. Cancer **22**:76, 1934.

A study of the glycolytic breakdown of various carbohydrates by tumor tissue shows the same sugars glycolyzed as are utilized by normal tissue. The anomalous aspect of the carbohydrate metabolism of malignant tissue springs from a difference in degree rather than in kind.

FROM THE AUTHOR'S CONCLUSIONS.

AMMONIA PRODUCTION BY SARCOMA: THE SPARING EFFECT OF THE CARBOHYDRATE. W. T. SALTER and P. D. ROBB, Am. J. Cancer **22**:87, 1934.

The utilization of various carbohydrate derivatives by mouse sarcoma 180 has been studied with respect to the lessening production of ammonia by the malignant tissue. The carbohydrates with which there is the most decrease in the production

of ammonia by sarcoma are those which are best glycolyzed. That liver shows no such effect is consistent with its low glycolytic index. Cyanide affects neither glycolysis nor the sparing of ammonia, but iodo-acetate checks both. The total of nonprotein nitrogen eliminated is unaffected by the presence of sugars which spare ammonia.

FROM THE AUTHORS' CONCLUSIONS.

ARSENICAL KERATOSES AND CARCINOMA. C. C. FRANSEEN and G. W. TAYLOR, Am. J. Cancer **22**:287, 1934.

Nine cases of carcinoma due to arsenic and five cases probably of arsenical origin are reported. Arsenic deposited in the skin may manifest its carcinogenic property as late as forty years after the ingestion of, or occupational exposure to, arsenic. The carcinogenic property of inorganic arsenic is not universally appreciated, and, as a result, carcinoma of the skin may inadvertently be produced. Inorganic trivalent arsenic, usually in the form of Fowler's solution (solution of potassium arsenite), appears to be the chief carcinogenic agent. Chronic arsenical lesions following the administration of organic arsenical compounds are exceedingly rare. Arsenical carcinomas are not invariably of the squamous cell type, as evidenced by the fact that more than one third of the carcinomas in this series were of the basal cell type. Although the malignancy of the squamous cell lesions is usually of low grade, metastasis to the groin and axilla is not infrequent, as attested in our series in nine lesions, two thirds of which were graded 1 histologically. With all lesions of considerable size, therefore, the regional lymph nodes should be removed, in spite of low histologic grading. Patients with early arsenical lesions may be spared extensive operations or untimely death by prophylactic destruction of precancerous arsenical keratoses; or, by careful and frequent observation, these keratoses may be destroyed at the moment malignant changes threaten.

FROM THE AUTHORS' CONCLUSIONS.

A NEOPLASTIC DISEASE OF THE KIDNEY OF THE FROG. B. LUCKÉ, Am. J. Cancer **22**:326, 1934.

Three of 276 leopard frogs with neoplastic disease of the kidney had tumors in organs distant from the kidneys: one, in the liver and bladder; a second, in the liver and left orbit, and a third in the liver. These tumors are believed to represent true metastases. The occurrence of such metastasis in cold-blooded vertebrates generally is discussed.

FROM THE AUTHOR'S SUMMARY.

ROFFO'S TEST IN CANCER: STATISTICAL RESULTS OF 11,000 CASES. A. GANDOLFO, Am. J. Cancer **22**:363, 1934.

Though Roffo's test is not specific, it is of value as an auxiliary method in the diagnosis of cancer. The technic is as follows: To 1 cc. of fresh, clear serum, 5 drops of 1 per cent neutral red in distilled water are added. If the serum assumes a red color, the reaction is regarded as positive. A yellowish hue indicates a negative reaction. The following precautions are to be taken: 1. The serum is not to be obtained by centrifugation; the blood must coagulate and the limpid serum be extracted by means of a suction pipet, the slightest trace of hemolysis altering the results. 2. The blood must be withdrawn before breakfast, in order to avoid the influence of nutritive lipoids. 3. Neutral glass tubes must be used, as alkalinity influences the results.

A negative test does not exclude the presence of a tumor; a positive test should induce one to continue investigations, in order to discover the tumor, as the proportion of erroneous positive results is small (6.37 per cent in 6,718 cases). The test has given a high percentage of positive results in cancer of the uterus (68.33 per cent), ovary (78.94 per cent), bladder (72.41 per cent), stomach (76.26 per cent), intestine (76 per cent), liver (84.20 per cent), pancreas (84.61 per cent), lungs (75 per cent) and mediastinum (86.66 per cent), and in osteosarcoma (71.42 per cent), all of which generally offer greater difficulties in clinical diagnosis.

FROM THE AUTHOR'S SUMMARY.

BENIGN NEOPLASMS OF THE RAT'S BREAST. J. HEIMAN, Am. J. Cancer **22**:497, 1934.

Benign fibroma and fibro-adenoma of the rat breast are easily and continuously transplantable. They grow, when transplanted, not only in the region of the mammary glands, but also in the axillae, groins, nape of the neck and outer side of the thigh and in the abdominal cavity. The transplanted tumors do not always retain the structure of the spontaneous tumors from which they are derived. The transplantability of a tumor is not a criterion of malignancy. Although the growth energy of these tumors fluctuates widely, there has been no cessation in one series for fifty-three generations, and in a fourth series for sixteen generations, during a period of ten years. They grow as readily in adult rats as in young rats. In the former the growth tends toward glandular hyperplasia, in the latter toward a marked increase of fibroblasts. Three of the six primary fibro-adenomas of the breast which were transplanted through four generations or more became actively growing cellular tumors with the morphology of sarcoma. Some of the tumors ulcerate through the skin, but this is due only to pressure on the skin and is not an evidence of malignancy. With a large number of inoculations and the implantation of two or more fragments 3 mm. in diameter, these benign tumors are readily transplantable for many generations in suitable hosts. The benign tumors as they develop into sarcoma require smaller and fewer fragments for transplantation. Dr. Otto F. Krehbiel has transplanted one such tumor by the trocar method, using 3 mg. of tumor substance, for fifty-six generations. Of sixteen rats with spontaneous benign tumors, six (37 per cent) yielded tumors transplantable for from four to fifty-three generations.

FROM THE AUTHOR'S SUMMARY.

TERATOMA OF TESTIS. C. C. HERGER and A. A. THIBAUDEAU, Am. J. Cancer **22**: 525, 1934.

The embryonal carcinomas with lymphoid stroma proved the most radiosensitive and yielded the most satisfactory clinical results. Experience with the quantitative Aschheim-Zondek reaction convinces us of its value as a diagnostic procedure and as an aid in the conduct of treatment.

FROM THE AUTHORS' SUMMARY.

THE CARBOHYDRATE TOLERANCE IN CANCER AND THE EFFECT OF ROENTGEN RADIATION. F. H. L. TAYLOR and H. JACKSON JR., Am. J. Cancer **22**:536, 1934.

In only one third of thirty-five patients with cancer was a decreased tolerance for dextrose found prior to the institution of roentgen therapy. Consideration must be given to the fact that a lowered tolerance for dextrose is not an uncommon tendency in persons between 55 and 70 years of age, particularly if they are suffering from malnutrition. The presence of a lowered tolerance for carbohydrate in patients with cancer is not diagnostic nor is it of assistance in prognosis. However, a progressively increased abnormality of the tolerance for carbohydrate may be regarded as an unfavorable sign. Roentgen radiation has no consistent effect on the sugar tolerance of cancerous patients. No relationship has been found between the decreased tolerance for sugar occurring in some patients with cancer and the total serum calcium.

FROM THE AUTHORS' SUMMARY.

ROUND-CELL, SPINDLE-CELL, AND NEUROGENIC SARCOMAS OF THE LIP. T. DE CHOLNOKY, Am. J. Cancer **22**:548, 1934.

In the available literature reports of 20 cases of sarcoma of the lip were found, in only 5 of which complete records were given. Four additional cases are reported: one of round cell sarcoma of the upper lip, two of spindle cell sarcoma and one of neurogenic sarcoma of the lower lip. In all cases the growth was on the vermilion border. In three of the cases the sarcoma was treated by wide

local excision followed by dissection of the regional nodes. The tumor on the upper lip was removed by electrocoagulation. The patients were all symptom-free after periods of six months (the one with round cell and one of the two with spindle cell sarcoma), eight months (the one with neurogenic sarcoma), and four years (one with spindle cell sarcoma). FROM THE AUTHOR'S SUMMARY.

METABOLIC STUDIES IN MOUSE LEUKEMIA. J. VICTOR and M. R. WINTERSTEINER, Am. J. Cancer **22**:561, 1934.

The oxygen consumption, aerobic glycolysis and anaerobic glycolysis of lymph nodes of normal mice and of those inoculated with different lines of transmissible lymphatic leukemia were measured in animals in which age and genetic constitution were controlled. There are metabolic differences between normal and leukemic lymph nodes. Inherent metabolic differences were observed not only between different lines of leukemia derived from the same organ in different spontaneous cases, but also between lines derived from different organs in the same spontaneous case. The metabolism of the individual line of transmissible leukemia was consistent during the period of observation. In no case was the oxygen consumption of leukemic nodes less than normal. The anaerobic glycolysis was always greater than normal. The results are statistically compared with others reported in the literature. Factors causing variations in results are discussed.

FROM THE AUTHORS' SUMMARY.

THE BEARING OF GENETIC WORK WITH TRANSPLANTED TUMORS ON THE GENETICS OF SPONTANEOUS TUMORS IN MICE. C. C. LITTLE, Am. J. Cancer **22**:578, 1934.

"The skepticism and criticism of genetic work by non-geneticists is no new development in the broad field of cancer research. Similar attitudes might be cited on the part of surgeons for radiologists, on the part of chemists for pathologists, and vice versa. The point seems to be that, as our knowledge of the complexity of cancer increases, greater intolerance by various specialized groups and individuals, for the work of specialists of other types, is needed. In the fight against cancer, experimental genetics certainly has its place. In the further development of cancer research, in all three levels of transplantable, induced, and spontaneous tumors, the genetic point of view will eliminate or analyze variables and will continue to make for accuracy and predictability of results."

THE DEVELOPMENT OF MULTIPLE TUMORS IN TARRED AND RADIATED MICE. M. C. REINHARD, A. A. THIBAUDEAU and C. F. CANDEE, Am. J. Cancer **22**:590, 1934.

As a result of one experiment involving the use of more than one hundred mice there is no evidence that short wavelength radiation changes the susceptibility of mice to the production of tar tumors, nor does the radiation alter the carcinogenic power of the tar used in this experiment. We believe that the low incidence of spontaneous tumors in the nonirradiated mice is a direct result of the tarring. In the irradiated group the low incidence may be attributed to the tarring also, or to the irradiation, or to a combination of both. Of course, it is possible that the occurrence of the spontaneous tumors has merely been retarded, and, had the mice lived longer, a percentage of mammary carcinomas might have been obtained which would more nearly approximate the normal expectancy. The appearance of the multiple tumors, distant in all cases from the site of painting, may be considered as evidence in favor of a general action of the tar. This is especially emphasized by the very striking absence of tumors at the site of painting. The possibility of chance contact must not be overlooked, but in view of the failure to produce tumors at the site of painting itself, this possibility is somewhat remote. On the other hand, we again point to the difference in the histologic picture as between the multiple sebaceous adenoma produced in this series and the typical tar cancer produced locally by repeated paintings with this agent.

FROM THE AUTHORS' SUMMARY.

A Study of the Serum of Chickens Resistant to Rous Sarcoma. F. G. Banting and S. Gairns, Am. J. Cancer **22**:611, 1934.

Serum resistant to Rous sarcoma neutralizes extract of Rous tumor; this neutralization occurs during incubation. The neutralizing power is destroyed between 70 and 80 C. Intravenous administration of the serum has no effect on a growing Rous tumor. Serum resistant to Fujinami sarcoma does not neutralize Rous tumor extract. Serum resistant to Rous sarcoma does not neutralize Fujinami extract.

From the Authors' Summary.

Rous Sarcoma Tissue Grafts in Susceptible and Resistant Chickens. D. Irwin, S. Gairns and F. G. Banting, Am. J. Cancer **22**:615, 1934.

In both susceptible and resistant birds all the tumor cells of Rous sarcoma sac grafts became necrotic. In susceptible birds the host cells surrounding the sac grafts gave rise to fatal tumors. In resistant birds small amounts of tumor-like tissue formed adjacent to the sac graft; this tissue failed to take on malignant characteristics and subsequently regressed. Cells and the tumor-producing substance in the sac grafts did not survive more than forty-eight hours in resistant birds.

From the Authors' Summary.

Nephrogenic Tumors. C. F. Geschickter and H. Widenhorn, Am. J. Cancer **22**:620, 1934.

In spite of the difficulty in demonstrating the remains of embryonic nephrogenic tissue in the more slowly growing malignant nephromas, and in the nephrogenic zone of the opposite kidney, comparisons of the Wilms tumor, the benign and malignant cystadenomas and the more rapidly growing malignant nephromas in young adults indicate that all of these nephrogenic tumors have their origin in the same mother substance. Judging from the microscopic studies, this tissue of origin is the renal blastema, a compact growth of spindle cells which differentiate into small tubular structures composed of oval epithelial-like cells. This type of primitive tissue predominates in the Wilms tumors in children, the spindle cells being more prominent than early tubule formation. In many of the benign and malignant cystadenomas of young adults, spindle cells and small tubules composed of oval epithelial cells predominate, particularly in those growths which have undergone malignant change. In the nephromas, occurring in old age, the blastemal elements are difficult to demonstrate, except in the more malignant and rapidly growing ones, but nevertheless they do occur. Taking the three groups of tumors as a whole, the shift from immature spindle to adult epithelial forms appears to represent the cycle of growth within the individual tumor, and the extent of maturity reached, as shown microscopically by scarcity or prevalence of larger epithelial elements, can be correlated definitely with the age incidence of the tumor and its relative degree of malignancy. The fact that all of these neoplasms show a distinct tendency to make their appearance just beneath the capsule in the normal growth zone of the kidney, and the fact that the cortical zone of renal tissue normally continues to grow until adult life, relate all of the growths to a single type of developing tissue. Hence the present study seems to indicate that the variations in structure which make for separate types of tumor are the expression of the various rates of growth and the extent of differentiation achieved by the individual form of tumor, rather than of their origin from separate and distinct tissues.

From the Authors' Summary.

Carcinoid Tumors of the Small Intestine. E. M. Humphreys, Am. J. Cancer **22**:765, 1934.

In a series of 3,200 cases in which autopsy was performed there were 3 of carcinoid tumor with metastases in regional lymph nodes. In 2 of these the carcinoids were multiple, one with 2, the other with 9 independent tumors. In 2

cases the lumen of the bowel was narrowed appreciably, and while the clinical evidence, though suggestive, was not conclusive, the anatomic features indicated a low grade intestinal obstruction. In the same series there were 5 cases of solitary carcinoid tumor without metastases, 1 in the jejunum, 3 in the ileum and 1 in the rectum (a carcinoid polyp). Thus in this series there was an incidence for all carcinoids of 0.25 per cent; for carcinoids of the small intestine, 0.22 per cent; for multiple carcinoids, 0.06 per cent, and for metastasizing carcinoids, 0.09 per cent. From a study of these cases and of case reports in the literature, it is evident that the carcinoid tumor of the small intestine is a far from harmless lesion. Of 152 tumors, 24.4 per cent had metastasized, and 24 per cent were responsible for symptoms of intestinal obstruction. The incidence of multiple carcinoids in the latter series was over 30 per cent. FROM THE AUTHOR'S SUMMARY.

THE RELATIONS OF TISSUE METABOLISM TO CONSTITUTIONAL PREDISPOSITION TO CANCER. B. WALTHARD, Ztschr. f. Krebsforsch. **40**:447, 1934.

Walthard has observed, as have others, that in degenerating tissues there is frequently a diminution of oxidative activity accompanied by an increase of aerobic glycolysis. As not nearly all changes of this sort are antecedent to cancer, he is inclined to question Fischer-Wasels' identification of this type of change with the appearance of the cancerous predisposition. In his opinion, the nature of this predisposition cannot be identified so simply, and still remains unknown.

H. E. EGGERS.

THE LONG-TIME TREND OF CANCER STATISTICS. S. PELLER, Ztschr. f. Krebsforsch. **40**:465, 1934.

Peller was among the first to suggest that cancer statistics, properly evaluated, show a diminution of the disease. Here he summarizes the results of such an evaluation of statistics from world-wide sources and reaches the conclusion that during the last twenty-five years there has been a real diminution in the frequency of cancer. This has been accompanied by changes in the relative frequency of primary sites, some of these showing a real increase. For males, he has no explanation to suggest for this shift, but for females he believes that diminished childbearing may be responsible. The general diminution cannot be explained by improved therapeusis, since it occurs very strikingly in such inaccessible cancers as those of the stomach and esophagus. The statistics of Vienna for the last few years indicate that here there has been a reversal of this trend, with an actual increase there of mortality from cancer; but even so, the actual (corrected) rate there is smaller than it was from twenty to thirty years ago. H. E. EGGERS.

THE SO-CALLED PERIPHERAL REACTION OF TUMORS AND THEIR METASTASES. J. R. M. INNES, Ztschr. f. Krebsforsch. **40**:527, 1934.

A very considerable series of malignant tumors, both primary and metastatic, were studied with a view to the reaction of the immediately surrounding tissues. The picture was in all cases essentially the same, consisting of lymphocytic infiltration along with that of plasma cells and more or less connective tissue overgrowth. Special features of reaction such as are occasionally seen with implanted tumors in animals Innes regards as being responses to the presence of material actually foreign to the new host. He could also detect no relationship of this peripheral reaction to prognosis.

H. E. EGGERS.

BASAL CELL CARCINOMA WITH CYST FORMATION. K. ENGLMANN, Ztschr. f. Krebsforsch. **40**:546, 1934.

Englmann reports six cases of basal cell carcinoma with the unusual feature of small cysts filled with mucus in the infiltrated epithelium. Five were primary on the face; the sixth was anal. Englmann believes that the cysts are not the

result of epithelial degeneration, but of cellular secretory activity. Unlike the usual rodent ulcers, these occur occasionally at early ages; two of the patients were aged 22 and 32 years, respectively. Ulceration of these tumors is absent or late, the tumor progressing as a deep-seated thickening of the skin. They run a slow course with indefinite infiltration, and may give late metastases.

H. E. EGGERS.

Technical

BLOOD WASSERMANN TEST IN NEUROSYPHILIS. W. C. MENNINGER and L. BROMBERG, J. Lab. & Clin. Med. **20**:698, 1935.

In approximately 30 per cent of cases of active neurosyphilis in which tests of the spinal fluid are positive the Wassermann reaction of the blood fails to give any indication of this process. In 23 per cent neither the Kahn nor the Wassermann test of the blood is positive. In view of this fact, syphilis, after the early stage, cannot in any case be regarded as completely studied, accurately diagnosed or correctly treated without knowledge of the cerebrospinal fluid.

FROM THE AUTHORS' CONCLUSIONS.

THE CALIBRATION OF GRADED COLLODION MEMBRANES. W. J. ELFORD and J. D. FERRY, Brit. J. Exper. Path. **16**:1, 1935.

Methods are described for calibrating graded collodion membranes by measurements of their thickness, their specific water content and the rate of flow of water through them and by calculation from these data of the average pore diameter. The several assumptions involved have been critically examined and evidence provided for their justification when applied to membranes of porosities greater than 10 microns. The relation of membrane structure and porosity is discussed, and a possible mechanism responsible for the gradation in the porosities of nitrocellulose films is advanced.

FROM THE AUTHORS' SUMMARY.

MECHANISING THE VIABLE COUNT. J. A. REYNIERS, J. Path. & Bact. **40**:437, 1935.

An effort has been made to mechanize the counting of viable bacteria. The thesis has been advanced that standardization of results can best be obtained by mechanizing the technic. To establish this thesis Reyniers has applied mechanical principles to what is now considered standard technic, i. e., the agar plate method, so that samples of cultures are treated in a uniform manner. The mechanized technic, called for convenience the R method, consists essentially of a mechanical distribution of bacteria on one plane and in a definite pattern on an agar surface. A comparison has been made between the R method, the standard method and the method of Wilson (roll tubes). The R method exhibits a lower percental mean deviation than either of the others. This would seem to indicate that a greater degree of standardization is possible through the use of mechanical principles which limit the technical errors and provide a uniform growing surface. The amount of growth obtained on an R plate is higher than is obtained with the standard or roll tube method. By comparison with other methods the R plate is easier to count because of the mechanical distribution of the colonies, and it permits the use of a mechanical counting device attached to a photo-electric cell. Considering the entire procedure of the R, standard and roll tube methods, the R method is easier to use and permits considerable saving of time. It is practical in field work because it allows the use of prepared agar disks, and can also be used in pure culture work.

FROM THE AUTHOR'S SUMMARY.

Society Transactions

PHILADELPHIA PATHOLOGICAL SOCIETY

March 14, 1935

MORTON MCCUTCHEON, *President, in the Chair*

AN UNUSUAL CASE OF CHRONIC TRACHEITIS AND BRONCHITIS. D. R. COMAN.

The case is that of a girl 2½ years of age who came to autopsy at the Hospital of the University of Pennsylvania. She was the seventeenth child of the mother. It was noticed early that she had dyspneic and wheezing respiration, especially when nursing. This difficulty increased and became particularly obvious when the child began to walk. When about 20 months old she caught cold and was ill for two weeks with fever and occasional cough which persisted and became worse. Four months later the child had real difficulty in getting breath, and at 27 months she was dyspneic even at rest. These spells came on three or four times a day and lasted for about an hour, during which the breathing was extremely labored. The condition was progressive.

At the time of admission to the hospital the child had paroxysmal coughing and wheezing musical râles on expiration, and retraction of the intercostal spaces and supraclavicular fossae on inspiration. Expiration was prolonged and the breath sound harsh. Her temperature rose from 98 to 101 F. A roentgenogram of the chest revealed considerable prominence of the lung markings and hili. The diagnosis was "high obstruction, cause uncertain." Bronchoscopic examination revealed granulation tissue which bled easily in the trachea and bronchi. Bacteriologic examinations gave negative results. The child's respiratory difficulty increased, and she died on Nov. 2, 1934.

At autopsy the mucosal surface of the trachea and bronchi presented a reddened, finely granular appearance with an overlying thin mantle of mucopurulent exudate. This lesion extended from the larynx through the trachea and along the bronchi as far as could be traced grossly. The lumens of the component parts of the respiratory tract were notably narrowed, and those of some of the bronchi were nearly occluded. Remarkable on gross examination was a peculiar increase in the cartilaginous elements of the bronchial walls. The cartilaginous plates were thicker and apparently extended farther out along the bronchial ramifications than normal. The lungs presented no other gross lesions.

The liver displayed on the broad anterior surface a few oat-sized yellowish-gray nodules. A few similar tiny nodules were found lying deep in the liver substance. The contents of these focal lesions were soft, almost caseous.

The rest of the body presented no significant departure from the normal.

The diagnosis at the time of autopsy was: exudative and productive tracheitis and bronchitis and chronic focal inflammation in the liver. The peculiar increase in the cartilaginous elements of the respiratory tree suggested a congenital anomaly rather than an acquired lesion, and the clinical history of early respiratory difficulty seemed to substantiate this view.

Microscopic preparations of the trachea showed the greater portion of the mucosal surface replaced by a heavy layer of granulation tissue composed of budding capillaries and proliferating fibrous connective tissue. There was a cellular exudate composed mostly of monocytes and polymorphonuclears. Beneath this layer remnants of ductal and glandular structures were seen.

Sections of the lungs showed the larger bronchi to be the seat of a chronic inflammatory reaction similar to that seen in the trachea. The most striking feature was the markedly thickened cartilage of the bronchi. The surrounding

lung tissue showed patchy atelectasis and emphysema, and many of the smaller bronchi and bronchioles showed a peripheral aggregation of lymphocytes, monocytes and plasma cells, while the mucosa remained essentially unaltered.

Microscopic section of the liver showed well circumscribed collections of exudative, cellular and proliferative inflammatory reaction not dissimilar from the reaction in the trachea and bronchi. The central portions of these foci contained densely packed cells of the large monocytic and plasma cell type surrounded by a zone of granulomatous reaction.

Stains for acid-fast organisms revealed none.

As regards a diagnostic classification of these lesions, much remains in the realm of speculation.

Aschoff (*Verhandl. d. deutsch. path. Gesellsch.* **14**:125, 1910) speaks of "tracheopathia chondro-osteoplastica," which he concludes is due to disease of the elastic bands of the trachea in which cartilage and bone are developed. He cited two cases, both in elderly men. In discussion of this, Steinberg stated that two forms of this lesion occur: one which Aschoff has described and a chronic inflammatory lesion. Chiari favored the existence of three types, and Schmorl concurred in this assumption: (1) a process secondary to chronic inflammatory lesions, (2) noninflammatory ecchondroses and (3) idiopathic noninflammatory new bone formation in the mucosa and submucosa.

The descriptions of cases by these and other men seem to refer more to an ecchondrosis than to a hypertrophy of the cartilage in its normal site, and their cases are apparently not of a character analogous to the case presented here, which, from its clinical history and from the results of gross and microscopic examination, suggests an underlying congenital hyperplasia of the normally situated cartilaginous plates with a secondary chronic inflammatory lesion on the basis of this malformation.

MINOR HEMAGGLUTININS. WILLIAM P. BELK.

The blood of a young man convalescing from acute infectious mononucleosis was found to contain (in addition to an iso-agglutinin of high titer) an auto-agglutinin, four specific hetero-agglutinins and heterohemolysins for the erythrocytes of the horse, sheep, rabbit and guinea-pig, as well as the rouleau-forming property.

The various characteristics of these several substances are described in detail.

The appearance of so many antibodies, including the heterophilic, in the same blood suggests the action of a nonspecific stimulus rather than that of the Forssman antigen.

It is suggested that infectious mononucleosis may be characterized not so much by the presence of heterophilic sheep agglutinins as by the appearance of a variety of antibodies.

DEGENERATIVE CHANGES IN BLOOD LEUKOCYTES. MAX M. STRUMIA.

Although in recent years the morphologic examination of blood has made great strides, especially through the more general use of the nuclear shift of the neutrophils, a great wealth of information is still generally ignored. In all toxemias certain degenerative changes take place in the circulating blood cells. In the neutrophils they are particularly constant and easily determined. For practical purposes, these lesions may be divided into alterations of the granules, alterations of the cytoplasm and nuclear changes. The most obvious alterations of the granules are increase in size accompanied by hyperchromatism and polychromatophilia. Later on, when cytoplasmic and nuclear changes occur, the granules become coarse, larger, polychromatic, and finally disappear. In the cytoplasm there is edema with progressive separation of colloids leading to formation of bald patches and vacuoles. In the nucleus there is coagulation necrosis followed by swelling. The changes in the granules referred to have been pointed

but more often under the term "toxic granules." However, early changes in the granules, hyperchromatism and polychromatophilia, may occur independent of any other lesion in the cell and seem to be independent of more serious degenerative changes, such as fusion, swelling, basophilia, etc. The rapid appearance or increase of the simple hyperchromatism and polychromatophilia of the neutrophilic granules and their rapid disappearance point to a reversible process. Until the nature of these changes is more definitely known, it seems desirable to use a more general and distinctive term.

Experimental work shows that the appearance of hyperchromatism and polychromatophilia in the granules is the earliest and most constant sign of toxemia, being present even in very mild types. These changes are best studied by hourly observations of the blood in patients who have received small doses of vaccine, especially typhoid vaccine, intravenously. These are followed by changes in the cytoplasm and nuclear degeneration, which is to be considered as an irreversible process indicating practically the death of the cell.

INFLAMMATION AND BACTERIAL INVASIVENESS. VALY MENKIN.

Powerfully necrotizing irritants produce, as a result of an increase in capillary permeability and of lymphatic damage, an extremely prompt reaction, perhaps best termed "fixation." By this process the area of injury is mechanically circumscribed and the dissemination of the irritant is prevented. Staphylococcus aureus is such a bacterial irritant. Aleuronat is a chemical irritant of similar potency. Mild irritants, on the other hand, produce only a delayed reaction, thus allowing relatively free penetration of the irritant into the circulation for a considerable interval of time. In such instances occlusion of the draining lymphatics often takes place as late as two days subsequent to the inoculation of the irritant. This type of irritant is exemplified by the hemolytic streptococcus. Another instance has been recently demonstrated by McMaster and Hudack, who showed that up to forty-eight hours following a mere incision of the skin or local burn lymph drainage was adequate. Subsequently the lymphatics failed to convey effectively the materials contained in them. The intensity of fixation is found frequently to be parallel to the extent of the inflammatory edema. This suggests that the local swelling is at least in part the result of blockage to the normal lymphatic drainage, which is thus unable to cope adequately with the excess outpouring of plasma from the capillaries at the site of inflammation.

In relatively large suppurating or acutely inflamed areas the reaction of fixation may occur as early as thirty minutes after the injection of an irritant. This prompt response allows a definite interval of time for the relatively sluggish leukocytes to assemble at the site of inflammation for the purpose of phagocytosis. The neutrophils appear first, to be displaced subsequently by the macrophages. This cytologic sequence, as recently demonstrated, is evidently conditioned by changes in the local hydrogen ion concentration. With the development of local acidosis at the site of acute inflammation predominance in the exudate shifts from the neutrophil to the mononuclear phagocytic type. It is conceivable that the mechanism of suppuration is closely associated, perhaps through an activation of autolytic tissue enzymes, with the local increase in the hydrogen ion concentration of an inflamed area. Studies are now in progress in an attempt to clarify this problem.

The reaction of fixation, by mechanically circumscribing the irritant in the earliest phase of the acute inflammatory reaction, plays a definite rôle in immunity, for it protects the organism as a whole at the expense of local injury. The reason for the disastrous effects resulting from untimely surgical intervention with such an effective inflammatory barrier as described and as encountered, for example, in the staphylococcus boil or the anthrax carbuncle is quite obvious and needs no particular emphasis in view of the foregoing discussion.

CHICAGO PATHOLOGICAL SOCIETY

April 8, 1935

I. Pilot, *Presiding*

Edwin F. Hirsch, *Secretary*

Necrosis of the Stomach Following Intravenous Injections of Neoarsphenamine. O. O. Christianson.

This article will be published in full in the Archives of Pathology.

DISCUSSION

P. R. Cannon: Do you think that arsenic entering the stomach in the regurgitated bile caused the changes in the gastric blood vessels?

V. Levine: Could the changes in the stomach have been due to the agranulocytosis? With that disease necrosis occurs in various tissues of the body.

O. O. Christianson: The injury to the blood vessels probably was caused by arsenic excreted from the blood. Necrosis of the lining of the stomach does not occur, so far as I know, with agranulocytosis.

Value of Chemical and Pathologic Observations in Pneumonoconiosis, with Special Emphasis on Silicosis and Silicotuberculosis. Henry C. Sweany, Jesse E. Douglass and Julius Porsche.

The antemortem and postmortem observations in forty-four patients most of whom had some form of pneumonoconiosis were correlated with the contents of total silica in the various parts of their respiratory tracts. With certain exceptions the total silica content varies roughly with the time and the type of exposure to silica. When there has been exposure to coarsely divided silica or silicates (sand or dust) in people having faulty physiology of the bronchial tract (bronchiectasis, emphysema, tuberculosis, etc.) the silica tends to be retained and produces changes in proportion to the amount of dust retained. Petrographic analysis determines the size and quality of such particles.

The silica content in people without silicosis usually varies from less than 0.5 mg. per gram of dried lung in the infant to between 1.5 and 2 mg. per gram of dried lung in the aged. The silica content of the lymph nodes of nonsilicotic persons rises from near zero in infancy to 1 mg. at 8 years of age and to 6 mg. in old age. In silicosis the silica content of the lymph nodes does not rise much above the amount found in nonsilicotic adults, because the nodes in the latter seem to be near the saturation point for the development of silicosis. This may help to explain the early appearance of specific whorls in the lymph nodes. When in people exposed to pure silica the silica content of the lung exceeds 2 mg. per gram of dried lung specific nodulation may be expected to develop. The silica content of the lung then rises roughly in proportion to the time and the amount of exposure to exceed the content found in the lymph nodes. This indicates that the lymph nodes soon become saturated, after which they take up silica only in diminishing amounts.

The silica content of the lung tissues may increase to 25 mg. per gram of dried lung or more, depending on the type of work and the interval of time in the work. The lungs of stone cutters, stone carvers and others exposed to such large quantities of silica have the highest. The silica content of coal miners' lungs may become double the high normal limit without showing specific nodulation. This may be due to the presence of silicates or, more likely, to the dampening effect of the carbon on the fibrosis. When a coal miner works in silica the lymphatics seem to be blocked and show no increase in silica content, while in the parenchyma it rises to high levels. This indicates not only blockage of the

lymphatics but also impairment of the bronchial and ciliary action. There is a moderate increase of the silica content of the pigmented portions over that of the nonpigmented portions. This indicates that the black portions have accumulated silica as well as carbon.

Probably the irritation due to tuberculosis causes an influx of silica-laden phagocytes from without. Any new growth or acute inflammation developing in such a lung will dilute the silica in proportion to the new cells introduced. Tuberculosis causes the development of nodules or masses that may be described as a tuberculosilicosis complex. A gross pathologic diagnosis of the disease silicosis is always possible, although nodules of silicotuberculosis may be mistaken for those of tuberculosis. Tuberculosis changes silicosis to a grave and fatal disease, sometimes terminating quickly in caseous pneumonia. Coal dust seems to retard silicosis, tuberculosis and silicotuberculosis, bringing about the development of large black masses, larger and more benign than the tuberculosilicotic complex.

The most important question yet to answer is how much the minimal silicotic nodulation predisposes to the tuberculous infection. Would these patients have died of tuberculosis (as many do) without the silicosis, or does the silicotic nodulation accentuate the tuberculosis? If we are to judge by the more advanced cases of silicosis and tuberculosis, every silicotic nodule is a menace, and every bit of finely powdered silica added to a tuberculous focus increases the tuberculous hazard. Some patients with slight involvement seem to carry considerable silicosis or modified silicosis without disability or impairment of health. There is evidence of individual variation among patients. There is also a difference of time at which disease appears, depending on the amount of silica, the amount of coal dust, the age of the patient and the state in which the silica exists. The diagnosis of the second stage of silicosis by means of the x-rays is relatively easy. In conditions that have not yet attained the second stage and any of the forms of modified silicosis (especially tuberculosilicosis) a diagnosis by means of the x-rays is as likely to be wrong as right. The clinical findings alone are of little use except to rule out other diseases. The history of exposure and the pathologic and chemical studies are the only certain means of diagnosis.

DISCUSSION

V. LEVINE: The micro-incineration method has been used to advantage to detect silicon in lymph nodes. I have in mind a patient who died of ulcerative tuberculosis. This patient had one large lymph node in the upper lobe of one lung, and by means of the micro-incineration method silicon was readily detected, whereas there was no silicosis in the remaining portion of the lung.

P. R. CANNON: Was there evidence of silicosis in the spleen, liver and bone marrow?

S. R. ROSENTHAL: Are the hilar lymph node tissues entirely destroyed in silicosis?

H. C. SWEANY: We now use a petrographic microscope which is valuable in differentiating silicon from other closely related doubly refractive substances. No silicosis was found in the livers or spleens of our patients with silicosis of the lungs. In many lymph nodes the lymphoid tissues are entirely destroyed, whorls of fibrous tissue are produced, and the lymph channels are blocked. In anthracotic lymph nodes the lymphoid tissue is destroyed to some extent, but no whorls are produced, and there is no blockage of the lymph channels.

FLUCTUATIONS IN BASOPHILIC AGGREGATION COUNTS WITH METEOROLOGIC ALTERATIONS. G. HOWARD GOWEN.

Daily basophilic aggregation counts were made on four normal young laboratory workers according to the McCord method during the entire month of October 1934. When the daily counts were expressed graphically there were daily fluctuations with definite peaks. In all four cases the peaks coincided rather accurately. When these graphs were compared with a meteorograph for October 1934 it was

found that the basophilic aggregation peaks occurred in most instances at those periods when the barometric pressure was high and the temperature low. Such weather conditions are termed "polar infalls," and it has been definitely shown that when they occur there is a blood pressor effect. These pressor episodes and their effects are reflected by the bone marrow in a moderate summation in the increased production of young cells. Inasmuch as the presence of basophilic substance has been interpreted as one of the most constant characteristics of immature red cells it is not unreasonable to assume that possibly the stimulation of bone marrow resulting from the pressor effects produced by the polar infalls results in an increased production and liberation of red cells exhibiting basophilic aggregations and in this way accounts for the daily fluctuations and peaks. In the preliminary basophilic aggregation counts made on persons entering an industry where there is a lead hazard the foregoing facts should be taken into consideration in evaluating the results of such counts.

BUFFALO PATHOLOGICAL SOCIETY

Joint Meeting with the Buffalo Academy of Medicine, April 24, 1935

KORNEL TERPLAN, *President, in the Chair*

W. F. JACOBS, *Secretary*

MASSIVE MUCOID CARCINOMA OF THE STOMACH. W. F. JACOBS.

A white man, 62 years of age, complained of pain on swallowing food, with relief on regurgitation, and of loss of weight and tarry stools. A bulging mass was palpated in the upper part of the abdomen extending inferiorly to 2 fingerbreadths above the umbilicus and blending laterally with the spleen and liver. Roentgenograms revealed narrowing of the lower end of the esophagus, irregularity of the cardiac end of the stomach and limited mobility. In a biopsy specimen obtained by esophagoscopy the stratified epiderm was acanthotic with scattered polymorphonuclear leukocytes, plasma cells and lymphocytes. In the subepithelial tissue could be made out an infiltrating mucoid carcinoma with signet ring cells and an abundant mucinous stroma. The patient died suddenly.

A finely granular gelatinoid ooze escaped from the abdominal cavity. The omentum was distinctly thickened. It was free, however, and covered coils of small intestine. The serosa of the small intestine was smooth and glistening. In the ileocecal area were situated two masslike accumulations of mucoid material. The lower end of the esophagus was converted into a narrow rigid tube. This portion of the esophagus and the fundus of the stomach formed the large mass which was palpated clinically. The stomach up to the antrum was a thick-walled rigid tube. The wall measured from 4 to 5 inches (about 10 to 13 cm.). The mucosa here was ulcerated. The serosa was covered with mucoid nodules.

The peritoneum covering the under surfaces of the diaphragm and the pancreas and spleen exhibited a mucoid tumor and was from ¼ to ½ inch (0.64 to 1.27 cm.) thick. No metastases were found in the lymph nodes or in the liver. Histologically the tumor was a mucoid carcinoma with signet ring cells and mucoid degeneration.

Death was due to pulmonary embolism.

The massive involvement of the stomach and the absence of metastases in the lymph nodes were considered noteworthy.

EFFECT OF INJECTION OF BILIRUBIN AND BILE SALT ON THE VAN DEN BERGH REACTION. NORMAN HEILBRUN and ROGER S. HUBBARD.

Pure bilirubin in amounts varying from 30 to 100 mg. was dissolved in tenth-molar sodium carbonate and injected intravenously. Direct van den Bergh reac-

tions were carried out on a specimen of plasma taken before the pigment was given (the control) and on specimens drawn five minutes after the injection. When small amounts of the material (from 30 to 50 mg.) were injected into normal subjects the reaction was invariably delayed, but larger amounts (from 75 to 100 mg.) produced an immediate reaction (color developing within from 10 to 30 seconds). When the pigment was injected into patients with severe hemolytic jaundice an immediate reaction was produced, although the reaction of the control was delayed.

In some instances a preparation of bile salt (20 cc. of a 20 per cent solution of sodium dehydrocholate) was injected either by itself or with the bilirubin. In four of five experiments this lessened the time before the appearance of the van den Bergh reaction or, if the reaction was already immediate, it significantly increased the intensity of the color produced.

Book Reviews

The Patient and the Weather. By William F. Petersen, M.D., with the assistance of Margaret E. Milliken, S.M. **Volume II. Autonomic Dysintegration.** Price, $5. Pp. 530, with 249 illustrations. **Volume III. Mental and Nervous Diseases.** Price, $5. Pp. 375, with 192 illustrations. Ann Arbor, Mich.: Edwards Brothers, Inc., 1934.

The publication of volumes II and III of Petersen's series of monographs on "The Patient and the Weather" has preceded that of volume I. Although the later volumes contain many references to presumably fundamental material in the first volume, each starts with a restatement of the author's concept and may be read independently of any other volume.

Forming the underlying groundwork of Petersen's concept is the view, now generally accepted, that the vascular system, especially its important peripheral bed, is in a constant state of change and flux. This vascular play may involve entire organs or larger areas of the body. It may and does constantly take place in the terrain of terminal arterioles; neighboring fields need not be in the same state at the same instant.

Stricker has made this mechanism and especially its state of vascular constriction or spasm the basis of much that makes up the field of pathology. For him the mechanism is a neurovasomotor one. Petersen goes much further and would make the rhythm of the vascular bed the result of many factors, both endogenous and exogenous, which mediate their effects through general or local chemical or physicochemical changes. In the biologic rhythm, the most obvious manifestation of which is vascular change, Petersen recognizes two antipodal states or phases, which, for the sake of brevity, he terms the ARS and COD phases. The first is characterized by tissue anabolism and alkalinity, tissue reduction and vascular spasm. These major alterations are associated with an increasing systolic pressure, increasing tissue acidity and capillary permeability, a decreasing carbon dioxide content, an increasing p_H and potassium-calcium ratio and an increasing cholesterol content. The ARS phase goes over gradually or abruptly into the COD phase. The tissues and vessels are stimulated by relative anoxemia. The author looks on the ensuing vasodilatation as an active process resulting from relative anoxemia; most pathologists have perhaps been in the habit of thinking of vascular dilatation as a more passive process. The COD phase is characterized by tissue catabolism and oxidation and vascular dilatation. These are associated with decreasing acidity and capillary and membrane permeability, a decreasing diastolic blood pressure, vascular dilatation, an increasing carbon dioxide and cholesterol content and a decreasing p_H and potassium-calcium ratio. These alterations are relative.

This interplay of phases is a fundamental manifestation of the living organism. It is a highly labile, autonomic and automatic mechanism that makes life possible. The person who is termed normal is attuned and adjusted to his vascular and tissue rhythm. In any local terrain excessive reactivity may exhibit results that an old-fashioned pathologist would be inclined to include under such heads as tissue stimulation, hyperplasia, degeneration, atrophy, fibrosis, necrosis and inflammation. The focal reaction may be a factor in bacterial localization and in the resulting chain of events that characterize infection. If the person is actually or potentially inadequate, the widest variety of abnormal physical or psychic states may result. All disease would appear to be traceable to abnormality of the biologic rhythm.

Although the rhythm is influenced by a wide variety of factors, both internal and environmental, the most important of these would appear to be the weather. Weather is not merely a matter of heat and humidity, which are the frequent subject of gibe and jest. It includes barometric pressure, velocity and direction of air currents, precipitation and electronic atmospheric ionization, as well as temperature and humidity. Of greatest importance are the brusque cyclonic changes that

reveal themselves in a change from a warm to a cold front or vice versa. "The human organism changes from day to day and . . . this change is related to the meteorological environment. . . . It [the human biologic mechanism] is a mechanism that must constantly be adjusted to myriad environmental influences, the chief ones being meteorological." Not only does the weather affect the individual from the time that he begins his more or less independent existence on this troubled earth following the severance of his umbilical cord, but it hounds him in his mother's womb and may cause malformation. It has been said that the best assurance for success in life is the proper selection of one's prospective parents. But one should do more than that. One should admonish one's future parents that they should exercise judgment in the performance of their marital rites and select a proper time for one's conception. Particularly if one is going to be obsessed by the ambition to be president of these United States he must urge his parents to be careful during August and September; February is the ideal month for breeding presidents.

Volume II "deals with the effects of meteorological alterations on the unstable individual. It includes a study of migraine, epilepsy, eclampsia, miscarriage, colitis, the sensitizations; the more or less fundamental problems of the mechanisms involved, the focal reaction, the importance of terminal vascularization, the rôle of anoxemia, etc." The volume begins with five opening chapters that have the following headings: "The Organism as a Cosmic Resonator"; "Vascularization"; "The Focal Reaction and Biological Rhythm"; "Penetration of Bacteria and the Localization of Disease"; "Focal Infection." Then follows a discussion of headache and migraine, with eleven detailed case studies. This is followed by chapters on epilepsy, eclampsia and premature delivery, mucous colitis and gastric ulcer, the neuroses, urticaria, asthma, arthritis, glaucoma, the ear, the teeth, alopecia areata and therapeutic implications.

Volume III "presents studies that have to do with mental and nervous disease." The eight chapters of the introductory first part have the following titles: "General Discussion"; "The Problem"; "The Focal Reaction in the Psychoses"; "Oxidation"; "Malformations"; "Mental Superiority—Insanity—Inferiority"; "Seasonal Conception"; "Suicide." Part II is devoted to a discussion and detailed case studies of certain psychoses, especially schizophrenia and the manic-depressive psychosis. Part III takes up multiple sclerosis, tabes and dementia paralytica, poliomyelitis and meningitis as nervous diseases in which the localization of the disease process is influenced by the focal reaction.

Each volume is illustrated by numerous graphic charts and maps. For each case presented in detail there is, in addition to numerous smaller graphs, a "comprehensive graph," the comprehensiveness of which interferes somewhat with its comprehension. Each graph has a meteorograph with six meteorologic factors. Beneath this the observations made on the subject are graphically presented. These observations, which may cover twenty or more different items, include, to select a graph at random, the reaction to the intracutaneous injection of epinephrine hydrochloride, the reaction to the intracutaneous injection of histamine, the dermographic reaction time, the McClure-Aldrich disappearance time, the systolic blood pressure, the diastolic blood pressure, the leukocyte count, the basal metabolic rate, the blood chlorides, the blood cholesterol, the blood sugar, the phosphates, the carbon dioxide content, the p_H, the calcium-potassium ratio, the potassium, the calcium, the serum protein and the blister time. The character of the observations made varies with the individual case. The use of the same unit to represent 1 mg. of one chemical substance and 10 mg. of another results in disproportionate magnification of the variations of those constituents that have a small amplitude of variation.

Petersen presents his material in a fascinating style that makes it difficult to lay aside a volume to gain the necessary sleep for the next day's work. To say that writing is fascinating is to bestow the highest praise. But the gentle art of book-reviewing demands the discovery of a fly in the ointment. So the reviewer turns entomologist, and perhaps even etymologist. This reviewer does not like, in a printed discourse, the abbreviation "T. B." for the disease tuberculosis, nor

does he think that it is conducive to international amity to refer to Japanese as "Japs." He can find no lexicographic warrant for "haemopoetic," although poiesis and poesy ultimately go back to the same Greek word meaning to make; or for "mucous" used as a noun; or for "neurophagia," "amytropic" and "hypospades," or "habitation" for habituation. "Albuminurea" is evidently a misprint for albuminuria. Schönlein, Strümpell, Lösung, Fällung are without the umlaut; if this is because the typewriter used in the preparation of the master script did not have this character, the difficulty could have been met by the use of the proper diphthong. The name of the reviewer's classmate is Loevenhart, not Loewenhardt. Eyes are described as "wide and starry"; the poet may speak of starry eyes, but for the physician eyes are staring. Some errors are grammatical in character, such as the use of a singular verb with a double subject and the use of "lead" as the past tense of to lead. "The fundi showed a salt and pepper fundus" may cause almost as much reaction in the leptosome reader as a polar infall. Petersen affects the prefix "dys" for dis. If the gastro-enterologist may write dyspepsia and the gynecologist dysmenorrhea, the author may be permitted to have a preference for "dysintegration," although this spelling does not have the sanction of usage.

It is not to be supposed that the examples just given have been sought out with malice aforethought and a fine-tooth comb. They have been encountered in the hurried survey that a reviewer makes of a book. There must be many more such flaws in what is otherwise delightful reading. The reviewer fears that they are evidence of carelessness.

Petersen's presentation of his wealth of material is a convincing statement of his thesis. After a volume has been laid aside and one has indulged in a little meditation and cogitation, one begins to wonder if an entire system of medicine or perhaps even of human biology is to be based on the weather. Döll of Berlin has recently presented a study in which, although he admits a correlation between terrestial meteorologic conditions and the state of the human body, he maintains that the latter is not dependent on the former, but that both the weather and the state of the body reflect electronic changes brought about on the earth by solar eruptions. If the weather-vane and the barometer are to replace the plexor and the stethoscope, perhaps after we have learned more about the effects of other planets on our own the conical cap of the astrologer will replace the mortarboard of the academician.

The author has presented the age-old conversational topic of the weather in a new and scientific manner. The books themselves present the now old art of printing in a new format. In the process, which is termed lithoprinting, a photographic electroplate of the typewritten page is made, and the inked impression is transferred to paper by means of a rubber roller. The reviewer has found the type much easier to read than the usual print of books or newspapers. The process lends itself well to the reproduction of illustrations and graphs.